

**Thermoregulatory capacity of arboreal small mammals in
the tropics: Insights from the past and implications for the
future**

By

SHAUN WELMAN

**Thesis submitted in fulfilment of the academic requirements for the degree of
Doctor of Philosophy**

In the Discipline of Evolutionary Physiology

School of Life Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg

South Africa

2018

Thesis abstract

Species are expected to respond to global warming through range shifts that are either poleward or towards higher altitude such that they track the movement of their current thermal niches. This generalized view is fundamentally flawed because it marginalizes the role of key biological aspects in shaping ecosystems. For example, it disregards any potential influence that phenotypic flexibility may convey, the influence of habitat heterogeneity and the availability of microclimates as thermal refugia, differences between species dispersal ability, the importance of species interactions, and the influence of phenological mismatches. Given the limitations associated with the view that species will deal with global warming by continually migrating, as well as the rapid rate of warming, there is an urgent need to improve the understanding of how species potentially may respond. Thus, it is crucial that mechanistic approaches are adopted to generate a holistic perspective of the factors that govern species distributions and use this information to forecast responses to global warming.

Physiological studies are vital because knowing the physiological tolerances of a species provides insight into their fundamental niche, and also provides a means of identifying species that face higher risks of experiencing more immediate effects due to global warming. Autecological knowledge would also serve to refine the species fundamental niche to more closely resemble their realized niche. In this regard, this thesis identifies arboreal mammals in the tropics as being vulnerable to hyperthermia due to global warming. The basis of this claim is related to the exposed lifestyle of many arboreal species, the biphasic effect of temperature on biological processes and the hypothesis that natural selection would have favoured the optimisation of bodily functions at or close to the species-specific body temperatures (T_b).

Initially, there is a positive relationship between biological process and temperature as the rate of processes increase with temperature, up to a maximum point. Thereafter, hyperthermia ensues as further increases in temperature results in a rapid decline in the rate of said processes. Thus, it is plausible to expect that species with lower T_b risk deleterious effects at lower absolute T_b s relative to their higher T_b counterparts. Therefore, it is concerning that many small, tropical endotherms have low and thermolabile T_b s that, because of the small temperature differential between themselves and the ambient (T_a), compromises their capacity to passively off-load excess stored body heat. In addition, the high humidity of tropical environments would theoretically reduce their capacity to retard heat storage by off-loading body heat via evaporation. This reduced capacity to dissipate excess stored body heat, in combination with the exposed life-style of an arboreal species, suggests that small, tropical arboreal mammals are vulnerable to hyperthermia should even minor increases in T_a occur.

The aim of this thesis was to assess the vulnerability of tropical, arboreal small mammals to hyperthermia due to global warming. This was achieved by determining and integrating the physiological susceptibility for heat stress in two species and relate that to the microclimate experienced within their habitats. Given the growing argument that adaptive heterothermy - the capacity for species to facultatively down-regulate metabolism and enter torpor or hibernation - may be employed at high T_a to cope better with hyperthermia, this thesis in addition investigated whether heterotherms use torpor at high T_a and identified the putative benefits of hyperthermic torpor. Furthermore, by considering the phylogenetic placement of the study species, this thesis also sought to provide insights into the evolution of endothermy in placental mammals.

Flow-through respirometry was used to measure resting metabolic rate (RMR) and evaporative water loss (EWL) at a range of T_a in a heterothermic bat, the lesser dog-faced fruit bat (*Cynopterus brachyotis*), and a suspected heterothermic primate, the western tarsier (*Cephalopachus bancanus*). The animals were injected with temperature-sensitive passive integrated transponder tags to obtain concurrent T_b readings during respirometry measurements in freshly-caught individuals. In addition, T_b was measured in free-ranging tarsiers using custom designed temperature data-loggers. The T_a at capture sites were measured using commercially available data-loggers.

The laboratory data show that, whereas tarsiers endeavoured to remain normothermic, lesser dog-faced fruit bats readily entered torpor at low temperatures. The free-ranging T_b data support the assertion that tarsiers may be incapable of adaptive heterothermy. The onset of heat storage in tarsiers occurred at approximately 30°C, once the thermal gradient ($\Delta T = T_b - T_a$) approached 4°C, whereas the onset of heat storage in lesser dog-faced fruit bats occurred at approximately 31°C, which was only when ΔT approached 1°C. Given that both species have low normothermic resting T_b s (tarsiers: $T_b \approx 34.5^\circ\text{C}$; lesser dog-faced fruit bats: $T_b \approx 32.5^\circ\text{C}$), they seem physiologically susceptible to heat stress at moderately low T_a . Notably, though, lesser dog-faced fruit bats appeared to thermoconform at T_a above their thermoneutral zone suggesting that they may have entered torpor. Torpor seems to have allowed them to reduce heat storage.

Field data suggest that lesser dog-faced fruit bats may have the option to exploit cool microclimates at their capture sites, but the data at the capture site of tarsiers suggest that they may not. However, even though the population of tarsiers studied may not have access to cool microclimates, the same may not be true for other populations of tarsiers. Thus, the empirical results support the argument that tropical, arboreal small mammals are physiologically susceptible to heat stress due to global warming, but they also suggest that

thermal refugia are an important consideration as they may allow species to escape the predicted future high T_a and its related deleterious effects.

This thesis also presents a meta-analysis on the thermoregulatory pattern of bats in general. The aim of this meta-analysis was to determine whether corroborating physiological support for the use of torpor at high T_a exists. A comprehensive literature search was conducted based on the availability of concurrent measures of T_b and RMR; a new dataset of thermoregulatory variables was generated for 29 species of bats (18 heterothermic spp. and 11 homeothermic spp.). The dataset was standardized, and phylogenetic relatedness was considered before any comparative analyses were performed. The results show that heterothermic bats maintain lower T_b s than homeothermic bats, yet they have similar upper limits of thermoneutrality (T_{uc}). In contrast to expectations, heterothermic bats had a lower rate of evaporative water loss at similar T_a , especially at T_{uc} . Crucially, in the case of heterothermic bats, T_{uc} exceeds T_b . The only manner in which heterothermic bats could achieve this, would be through a reduction in metabolic rate with the onset of heat storage at high T_a s. Moreover, heterothermic species thermoconform even at comparatively moderate T_a , which presumably also minimizes heat storage and lowers evaporative water loss. Thus, these results support the hypothesis of torpor use at high T_a and suggest that heterotherms, in particular small, tropical, arboreal heterotherms, could benefit from a reduction in water use associated with evaporative cooling and tolerate higher T_a .

Overall, the results presented in this thesis illustrate that even though small mammal species living in tropical regions may be physiologically susceptible to heat stress due to global warming, they could minimize their risk of lethal hyperthermia through behavioural mechanisms such as exploiting cooler microclimates; provided that suitable habitats are available. In addition, adaptive heterothermy may convey a physiological advantage that allows heterotherms to better cope with heat. As such, heterotherms may be resilient to the negative effects associated with global warming because they are able to employ torpor to conserve energy during periods of low resource availability, as well as to minimize heat storage and endure moderate hyperthermia to conserve water to use at more extreme T_a s. Given the phylogenetic placement of tarsiers at the base of the Haplorrhini clade (Anthropoidea and Tarsiidae), the current lack of evidence for adaptive heterothermy in tarsiers, in combination with the lack of evidence in anthropoids, suggest that adaptive heterothermy in haplorrhine primates may have been lost at the Strepsirrhini-Haplorrhini split. The implication of the aforementioned idea is that many primates may not have the benefits associated with adaptive heterothermy to improve their future survivability as global warming continues.

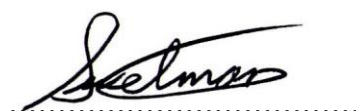
By adopting a mechanistic approach, this thesis highlighted the potential for species to respond to global warming using behavioural and physiological mechanisms that could allow them to persist in their current habitats until the end of the century at least. However, to improve the likelihood of arboreal tropical small mammals to persist in their current habitats for the foreseeable future, especially those mammals that are strictly homeothermic, conservation efforts must prioritise the preservation of areas that could serve as thermal refugia.

Preface

The data described in this thesis were collected in Borneo, Malaysia, between August 2014 and September 2015. The research contained in this thesis was completed by the candidate while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa, under the supervision of Professor Barry G. Lovegrove. Procedures involving the use of animals were reported to and approved by the University of Kwazulu-Natal Animal Ethics Committee (116/13/Animal). All experimental protocols were also approved by the Sarawak Forestry Department [permit number: NCCD.907.4.4(9)-223, NCCD.907.4.4(13)-277] and comply with national Malaysian laws.

The research was support by the National Research Foundation (South Africa), GreenMatter fellowship (South Africa) and their partners, as well as the Ministry of Higher Education of Malaysia.

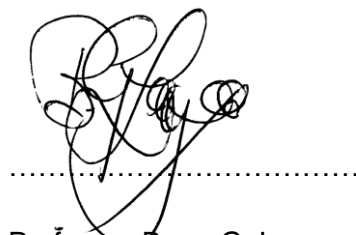
This thesis, submitted for the degree of Doctor of Philosophy in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any university. Where use has been made of the work of others, it is duly acknowledged in the text.



Shaun Welman

March 2018

I certify that the above statement is correct.




Professor Barry G. Lovegrove (Supervisor)

March 2018

Declaration 1: Plagiarism

I, Shaun Welman, declare that:

- I) The research reported in this thesis, except where otherwise indicated or acknowledged, is my original research;
- II) This thesis has not been submitted in full or in part for any degree or examination to any other university;
- III) This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;
- IV) This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
 - a) Their words have been re-written but the general information attributed to them has been referenced;
 - b) Where their exact words have been used, their writing has been placed inside quotation marks, and referenced;
- V) Where I have used material for which publications followed, I have indicated in detail my role in the work;
- VI) This thesis is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;
- VII) This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.



.....

Shaun Welman

2018

Declaration 2: Publications

This thesis is formatted as a series of manuscripts for which I am the primary investigator and lead author. Chapter 1 is formatted according to the “Perspectives” style of the journal *Evolution*. Chapters 2, 4 and 5 are formatted according to the “Research article” style of the journal *Frontiers in Physiology*. Chapter 3 is formatted according to the “Research article” style of the journal *Climate Research*. Because the chapters are written as individual manuscripts there is some inevitable repetition between chapters.

Publication 1

Welman, S. (in prep). Southeast Asia: An evolutionarily insightful but rapidly deteriorating ecosystem.

Publication 2

Welman, S., Tuen, A. A. and Lovegrove, B. G. (2017). Searching for the Haplorrhine Heterotherm: Field and Laboratory Data of Free-ranging Tarsiers. *Front. Physiol.* 8, 745, doi: 10.3389/fphys.2017.00745.

Author Contributions

S.W. and B.G.L. conceived and designed the study. S.W. performed the data collection, analyses and drafted the manuscript. B.G.L. contributed to and approved the manuscript. A.A.T. aided in securing capture permits, provided logistical support, as well as approved the manuscript.

Publication 3

Welman, S., Tuen, A. A. and Lovegrove, B. G. (2017). Using thermoregulatory profiles to assess climate change vulnerability in an arboreal tropical bat: Heterothermy may be a pre-adaptive advantage. *Clim. Res.* 74:161-170, doi: <https://doi.org/10.3354/cr01496>.

Author Contributions

S.W. and B.G.L. conceived and designed the study. S.W. performed the data collection, analyses and drafted the manuscript. B.G.L. contributed to and approved the manuscript. A.A.T. aided in securing capture permits, provided logistical support, as well as approved the manuscript.

Welman, S. & Lovegrove, B.G. (in prep). Torpor at high temperatures? Thermoregulatory comparison between heterothermic and homeothermic bats.

Author Contributions

S.W. and B.G.L conceived the study. S.W. designed the study, conceptualized and developed the model predictions, performed the data collection, data analyses and drafted the manuscript. B.G.L. provided advice about the data analyses as well as contributed to and approved the manuscript.

Original contributions to knowledge

Chapter one – Southeast Asia: an evolutionarily insightful but rapidly deteriorating ecosystem

The evolutionary history of mammals' remains hotly contested and the uncertainty has limited our framework of understanding regarding their evolution. There is now robust support for the argument that placental mammals evolved in warm tropical conditions because rainforests are thought to have evolved during the mid-Cretaceous (ca. 100 mya). Thus, placental mammals either evolved coincidentally with rainforests or would have evolved from an ancestor that already lived in a rainforest environment. Based on this framework, I argue that Southeast Asian mammals could provide valuable insights into the placental ancestor because they are likely to have retained plesiomorphic traits through stabilising because they have remained in tropical conditions since their evolution. This framework would be beneficial for evolutionary biologists and I provide an example of how it could facilitate studies that test hypotheses regarding the evolution of placental mammals using the evolution of endothermy as an example. There is urgent need to exploit the opportunity to study Southeast Asian mammals because the Southeast Asia region is under severe threat due to human-related impacts such as land use change and global warming. Thus, I argue that strategic studies could yield data that are vital for assessing the potential of Southeast Asian mammals to survive global warming and simultaneously document information that this evolutionarily insightful.

Chapter two - Searching for the Haplorrhine Heterotherm: Field and Laboratory Data of Free-ranging Tarsiers

Primates display a puzzling dichotomy in endothermic traits. Whereas adaptive heterothermy is wide-spread throughout the Strepsirrhini (lemurs, galagos and lorises), there

is no record of it within the sister clade, the Haplorrhini. There has been a recent paradigm shift in our view of the evolution of endothermy in mammals. The earlier paradigm was that strict homeothermy evolved directly from ectothermy. Thus, adaptive heterothermy was viewed as an apomorphic condition. The contemporary paradigm is that adaptive heterothermy is the plesiomorphic condition that served as the intermediate state between the ectothermic condition of the ancestral mammaliaforms and strictly homeothermic modern mammals. Given that tarsiers are basal in the clade Haplorrhini, their phylogenetic placement means that they are excellent candidates in which to search for adaptive heterothermy within this clade. Current physiological data for tarsiers are limited to a few metabolic rate measurements and potentially inaccurate skin temperature measurements of Philippine tarsiers (*Tarsius syrichta*). In this chapter, I present the first comprehensive description of thermoregulation in a tarsier other than *T. syrichta*, namely *Cephalopachus bancanus*. The data presented includes novel, concurrent, laboratory measurements of metabolic rate, body temperature and evaporative water loss, as well as core body temperature measurements in free-ranging individuals. The results of my study contradict earlier work and show that tarsiers may be highly susceptible to heat stress, even at moderate temperatures. Combining their thermoregulatory capacity, the microclimate data of their habitat and their exposed arboreal lifestyle, tarsiers may be vulnerable to the effects of global warming. This observation supports the argument that tropical arboreal endotherms may be physiologically vulnerable to global warming because they generally have low body temperatures and are exposed to environmental conditions that compromise their capacity to dissipate excess heat. However, the true novelty of this chapter is that it serves to further our understanding of the evolution of endothermy within primates by synthesizing all relevant work on haplorrhines and contextualizing the current lack of evidence for adaptive heterothermy in the Haplorrhini within our contemporary understanding of the evolution of endothermy. This chapter introduces a new, testable, hypothesis that adaptive heterothermy in Haplorrhini was lost at the Strepsirrhini-Haplorrhini split, and this hypothesis provides a framework for future physiology-based primate studies.

Chapter Three - Using thermoregulatory profiles to assess climate change vulnerability in an arboreal tropical bat: heterothermy may be a pre-adaptive advantage

The role that frugivorous and nectivorous bats play in maintaining ecosystem integrity is greatly underappreciated; especially within the context of global warming. On a global scale, the contribution of bats to plant pollination and seed dispersal is considered secondary to, for example, invertebrates and birds. However, within the tropics, bats arguably have the most important contribution to animal-plant interactions necessary to maintain the ecosystem integrity. In this chapter, I present an updated account of the thermoregulatory capacity of the

dog-faced fruit bat (*Cynopterus brachyotis*), a bat that is prominent within the bat-fruit network in the tropics. Unlike the initial physiological study on *C. brachyotis*, I present comprehensive thermoregulatory data illustrating variations in core body temperature directly, instead of variations in skin temperature that serve to approximate core temperature. I also reported the first account of the species' capacity for evaporative heat loss. As in Chapter 2, the species' thermoregulatory capacity is integrated with their habit microclimate data. The results show that *C. brachyotis* maintain a T_b that is much lower than previously reported and consequently they would seem to be, theoretically, more susceptible to heat stress due to global warming than initially thought. However, unlike the tarsiers studied in Chapter 2, *C. brachyotis* have the capacity to enter torpor at high ambient temperatures and appeared to thermoconform at both low and high temperatures. This observation adds to the evidence that heterothermic species may down-regulate metabolism at high temperatures and thermoconform to better cope with the heat (investigated in Chapter 4). Metabolic down-regulation at high T_a thus offsets the onset of heat storage. Given the benefits of torpor, the generalist nature of the species, as well as the cool microclimate of their habitat, I argue that *C. brachyotis* may be less vulnerable to the effects of global warming than expected based only on their thermoregulatory capacity and predicted increases in regional ambient temperature. As such, this chapter provides evidence that reaffirms the importance of integrating behavioural aspects, physiological principles and the experienced environmental conditions when evaluating a species response to global warming. Importantly, given the limited time and resources available to researchers and conservationists involved with climate change, this chapter provides an example of a framework of how to identify species whose survival are critical to ensure general ecosystem health and prioritizing conservation efforts accordingly.

Chapter Four - Torpor at high temperatures? Thermoregulatory comparison between heterothermic and homeothermic bats.

There is a growing argument that heterotherms are able to better cope with heat than homeotherms because they may use torpor at high temperature. Until now, there has been little corroborating physiological evidence to support the few claims that heterothermic species do down-regulate metabolism at high temperature. Chapter 4 builds on the initial arguments made in Chapter 3 and reviews the thermoregulatory capacity of bats to determine whether there are notable differences between heterothermic and homeothermic species. I present a new perspective on how to interpret the thermoregulatory profiles of heterothermic species and I also introduce the first formal attempt to test the hypothesis that heterothermic species use torpor at high temperatures. All contemporary models used to determine torpor expression fail at high temperature. Thus, I developed a new model that uses an indirect approach to determine whether metabolic down regulation does occur at high temperature. To do so, I

developed a new, standardized, dataset of thermoregulatory variables to address the systematic errors within existing datasets of physiological variables. Given that the results support the use of torpor at high temperature, I also integrated principles from molecular physiology to introduce a mechanistic framework of how metabolic down-regulation at high temperature may occur and then developed testable predictions that future work can use to further our understanding of torpor at high temperature. Aside from introducing a novel perspective on mammalian thermoregulation, the work conducted in this chapter builds on the argument that heterotherms have a pre-adapted advantage when dealing with the effects of global warming and using torpor at high temperature could have wide implications regarding species survivability and the likelihood that they will migrate, which has cascading implications for conservation planning.

Dedication

My thesis is dedicated to my parents Godfrey and Sybil Welman,
and to my brother Marcel Welman,
who supported and encouraged me throughout my academic career.
Thank-you for being pillars in my life.

Table of Contents

Thesis abstract.....	i
Preface	v
Declaration 1: Plagiarism	vi
Declaration 2: Publications.....	vii
Original contributions to knowledge.....	viii
Dedication.....	xii
Table of Contents.....	xiii
List of Figures	xvi
List of Tables	xix
Acknowledgments.....	xxi
Chapter one – Southeast Asia: an evolutionarily insightful but rapidly deteriorating ecosystem	1
Recent insight into the evolution of placental mammals.....	1
The potential for Southeast Asian mammals to provide meaningful insight into the placental ancestor	4
The value of Southeast Asia as a model system to test evolutionary hypotheses regarding the evolution of endothermy in placental mammals	5
A race against time: The threat of habitat loss and global warming in the tropics	7
Conclusion	10
Problem statement and Thesis rationale.....	10
Research objectives, study species and approach	11
Literature cited.....	14
Chapter Two - Searching for the Haplorrhine Heterotherm: Free-ranging Tarsier Data	26
Abstract.....	26
Introduction	27
Materials and methods	29
Animal capture and husbandry	29
Gas exchange measurements.....	30

Experimental protocol for gaseous exchange measurements.....	31
Surgical procedure and free-ranging body temperature measurements.....	32
Data analyses.....	33
Results	34
Thermoregulatory measurements.....	34
Free-ranging body temperature profile.....	38
Discussion	43
Conflict of interest statement	47
Author contributions.....	47
Funding	48
Acknowledgements	48
References.....	48
Chapter Three - Using thermoregulatory profiles to assess climate change vulnerability in an arboreal tropical bat: heterothermy may be a pre-adaptive advantage	59
Abstract.....	59
Introduction	60
Materials and methods	62
Study site and animal capture	62
Gas exchange measurements and experimental protocol	63
Data analyses.....	65
Results	66
Discussion.....	72
Acknowledgements	74
Literature cited.....	74
Chapter Four - Torpor at high temperatures? Thermoregulatory comparison between heterothermic and homeothermic bats.	81
Abstract.....	81
Introduction	82
Modeling hyperthermic torpor	84
Hyperthermic Torpor Model predictions	90

Materials and methods	94
Dataset.....	94
Calculating thermal limits.....	95
Phylogenetic analysis	95
Results	97
Phylogenetic signal and body mass.....	97
Normothermic body temperature and thermal limits.....	97
Body temperature and evaporative water loss at thermal limits	101
Discussion	105
Conflict of interest statement	109
Author contributions.....	109
Acknowledgements	109
Funding	110
Literature cited.....	110
Chapter Five – Thesis summary and future recommendations.....	119
Vulnerability of tropical mammals to heat stress due to climate change.....	119
The benefits of heterothermy during heat stress.....	120
The evolution of endothermy in mammals	122
Limitations of the current study and recommendations for future work	123
Literature cited.....	124

List of Figures

- Figure 1-1: The overlap between the evolutionary history of endothermy in mammals according to Lovegrove (2017), the origin of the placental ancestor and the origin of rainforest environments. The respective date of divergence of the superordinal groups of placental mammals are depicted according to O'Leary et al. (2013) and Foley et al. (2016), and the proposed origin of rainforest environments is according to Couvreur and Baker (2013). The time periods under the cross-hatch shading are not to scale. The asterisk represents the proposed origin of the placental ancestor. The palm silhouette represents the proposed origin of rainforests at approximately 100 mya, and was obtained from silhouettesfree.com under a Creative Commons License. The dashed line represents the Cretaceous-Paleogene (K-Pg) boundary. 3
- Figure 1-2: The phylogenetic relationship of the mammalian Orders within the clade Euarchontoglires. The date of divergence at each node is based on the long fuse model of Foley et al. (2016). The asterisk represents the date of divergence between the Euarchontoglires and Laurasiatheria superordinal groups. The mouse, rabbit, tree shrew, tarsier and flying lemur animal silhouettes depict commonly recognizable animals within their respective Order. Animal silhouettes were obtained from Phylopic.org under a Creative Commons License. The dashed line represents the Cretaceous-Paleogene (K-Pg) boundary. 5
- Figure 2-1: The thermoregulatory profile of *Cephalopachus bancanus*. a) Effect of ambient temperature on resting metabolic rate. b) Effect of ambient temperature on subcutaneous temperature. c) Effect of ambient temperature on the rate of evaporative water loss. d) Effect of ambient temperature on the amount of metabolic heat dissipated through evaporative cooling. Plotted regression lines indicate the best fit for the data based on piecewise regression analysis and the equations are presented in Table 2-1. Closed circles = males, open circles = females. 35
- Figure 2-2: The temperature profile in free-ranging western tarsiers (*Cephalopachus bancanus*). A) The ambient temperatures (T_a ; grey line) and core temperatures (T_{core} ; black line) recorded in a female from 00:00 24th June 2015 to 00:00 27th July 2015. B) The ambient temperatures (T_a ; grey line) and core temperatures (T_{core} ; black line) recorded in a male from 00:00 24th June 2015 to 06:30 10th July 2015. The grey and white bars represent night and day respectively, and the black bars at the base of each plot represent the temperature differential between the T_a and T_{core} 39
- Figure 2-3: The frequency distribution of the ambient temperatures (T_a) and core temperatures (T_{core}) recorded in free-ranging western tarsiers (*Cephalopachus bancanus*). A) The

distribution of temperatures corresponding to the female tarsier in Figure 2-2A. B) The distribution of temperatures corresponding to the male tarsier in Figure 2-2B.	40
Figure 2-4: The frequency distribution of the scotophase and photophase ambient temperatures recorded within the forest at Sama Jaya Nature Reserve from 00:00 24 th June 2015 to 00:00 27 th July 2015. The arrow indicates the commonly predicted shift of three degree Celsius in the modal temperature associated with global warming. .	42
Figure 3-1: The thermoregulatory profile of <i>Cynopterus brachyotis</i> . a) Effect of ambient temperature (T_a) on resting metabolic rate. The dashed line indicates basal metabolic rate (BMR). b) Effect of T_a on core body temperature (T_b). The diagonal line indicates where body temperature equals ambient temperature ($T_b = T_a$). c) Effect of ambient temperature on the rate of evaporative water loss (EWL). d) Effect of T_a on the amount of metabolic heat dissipated through evaporative cooling i.e. the ratio of evaporative heat loss (EHL) to metabolic heat produced (MHP).	67
Figure 3-2: Ambient temperature (T_a) recorded at capture sites between 18 February 2015 and 15 June 2015. Capture site 2: eastern slope of Mount Singai; capture site 3: Universiti Malaysia Sarawak campus. Data are presented as a percentage of the total number of recordings.	70
Figure 4-1: A schematic representation of the thermoregulatory response of an endotherm while defending a normothermic body temperature (T_b); adapted from Scholander et al. (1950), Lovegrove et al. (1991a), Tattersall et al. (2012) and Fristoe et al. (2015). a) The metabolic response of an endotherm to varying ambient temperatures (T_a s). The green and yellow shaded sections represent the range of T_a s and metabolism within the thermoneutral zone (TNZ) due to intra-specific seasonal phenotypic plasticity in thermal conductance (the slope of the metabolic response to T_a beyond TNZ) and basal metabolism (BMR, upper and lower dashed lines). The dark-blue and red shaded sections represent the range of metabolic rates required to defend normothermia. The light-blue section illustrates the potential influence of evaporative cooling on thermal conductance at high T_a s. The drop-lines are colored coded to indicate the corresponding T_a at the lower (T_{lc}) and upper (T_{uc}) limits of the TNZ. The grey shaded section represents the range of metabolic rates and T_a s over which heterotherms typically employ torpor or hibernation, that is, the abandonment of normothermia. b) The effect of evaporative cooling on the T_b profile of an endotherm defending normothermia. The red line represents the likely T_b profile in the absence of evaporative cooling, whereas the black line represents the T_b profile associated with moderate evaporative cooling and the light-blue line represents the T_b profile with effective evaporative cooling. The horizontal dashed lines indicate the level of the	

normothermic (lower) and lethal (upper) T_b s respectively. The dark-blue drop-line indicates T_{lc} and the drop-lines indicating T_{uc} are color coded to the T_b profiles. 86

Figure 4-2: A schematic model of the theoretical difference in the upper critical limit (T_{uc}) of the thermoneutral zone between endotherms that have similar capacities for thermal conductance and evaporative cooling, but one has a low (blue line) normothermic body temperature (T_b) and the other has a high normothermic T_b (red line). The model illustrates that the thermal gradient which facilitates passive heat dissipation, i.e. $T_b - T_a$ becomes unfavorable for heat loss at a relatively lower ambient temperature, resulting in an earlier onset of heat storage with increasing T_a 89

Figure 4-3: Theoretical models of the thermoregulatory adjustments in body temperature (T_b), upper critical limit of thermoneutrality (T_{uc}) and evaporative water loss (EWL) predicted by metabolic down-regulation in heterotherms at high ambient temperatures (T_{as}). The dark-blue line represents the T_b profile of heterotherms, the brown line represents the T_b profile of homeotherms, the black line represents a common T_b profile between homeotherms and heterotherms a) The predicted upward shift in T_{uc} (black arrow) by heterotherms to a higher T_a due to metabolic down-regulation reducing the animal's internal heat component and facilitating similar T_b s (indicated by the dashed line) at higher T_a s without the influence of evaporative cooling. b) The predicted shift in the T_b profile (black arrows) of heterotherms due to metabolic down-regulation reducing the animal's internal heat component and facilitating lower T_b s at similar T_{uc} s without the influence of evaporative cooling. c) The predicted lower rate of EWL by heterotherms (dark-blue open arrow) relative to homeotherms (brown open arrow) at similar T_b s due to metabolic down-regulation reducing the animal's internal heat component and thus requiring a lower rate of evaporative cooling to maintain a given T_b . The light-blue shade indicates the range of potential rates of EWL. 92

Figure 4-4: The phylogenetic relationship of bats used in the study and their relative body temperatures at the lower (left tree) and upper (right tree) critical limit of thermoneutrality. The phylogenetic tree is based on the phylogeny published by Shi and Rabosky (2015). The T_b range is plotted on a log scale and color coded from low (blue) to high (red). * indicates heterothermic species. 98

Figure 4-5: Box-and-whisker plots of the calculated body temperatures for the heterothermic ($n = 18$) and homeothermic ($n = 11$) bats species used in the study at a) the lower critical limit and b) the upper critical limit. The horizontal line in each box is the median value, the grey boxes define the 25th-75th percentiles, the whiskers define 10th-90th percentiles and black circles represent values beyond those percentiles. 102

Figure 4-6: Mean \pm standard error of homeothermic and heterothermic bat body temperatures at a range of ambient temperatures. The numbers above and below the plots represent the sample size (n) of homeothermic species and heterothermic species per temperature respectively. Only points with n > 2 species are reported..... 107

List of Tables

Table 2-1: Linear regression models describing the relationship between ambient temperature (T_a) and various physiological parameters in <i>Cephalopachus bancanus</i> . Mass-specific resting metabolic rate = RMR, subcutaneous temperature = T_{sub} , the rate of evaporative water loss = EWL, the amount of metabolic heat dissipated through evaporative cooling = EHL/MHP, * = $p < 0.05$, ** = $p < 0.01$	36
Table 2-2: Linear mixed-effect models which best describe the significant relationships between ambient temperature (T_a) and resting metabolic rate (RMR), subcutaneous temperature (T_{sub}), the rate of evaporative water loss (EWL) and the amount of metabolic heat dissipated through evaporative cooling (EHL/MHP) in <i>Cephalopachus bancanus</i> . Only models with Akaike weights ≥ 0.01 are presented.....	37
Table 2-3: The daily mean, maximum and minimum ambient (T_a) and tarsier core (T_{core}) temperatures observed throughout the period of free-ranging data collection (values in parenthesis represent the range in daily means), as well as the times at which these parameters were most frequently observed. Photophase corresponds to the animals rest phase, whereas scotophase corresponds to their active phase. ♂ = male, ♀ = female, max = highest temperature, min = lowest temperature, n = periods of observations and † indicates a significant ($p < 0.05$) Rayleigh's test.	41
Table 3-1: The relationship between ambient temperature (T_a) and mass-specific resting metabolic rate (RMR), body temperature (T_b), the rate of evaporative water loss (EWL) and the amount of metabolic heat dissipated through evaporative cooling (EHL/MHP) in non-torpid <i>Cynopterus brachyotis</i> . ** $p < 0.01$	68
Table 3-2: Best-fitting linear mixed-effect models evaluating the influence of ambient temperature (T_a), body mass (M_b), sex and capture site on resting metabolic rate (RMR), body temperature (T_b), the rate of evaporative water loss (EWL) and the amount of metabolic heat dissipated through evaporative cooling (EHL/MHP) in non-torpid <i>Cynopterus brachyotis</i> . k = the number of parameters for the model. AICc = Akaike's information criterion values corrected for small sample size. Only models with Akaike weights >0.01 are presented.....	71

Table 4-1: The body mass (M_b), normothermic body temperature (T_b), lower critical temperature (T_{lc}), upper critical temperature (T_{uc}), body temperature at the lower critical temperature (T_b at T_{lc}), body temperature at the upper critical temperature (T_b at T_{uc}), the difference in body temperature between the lower and upper critical temperatures (ΔT_b), and the species' thermoregulatory classification for the bat species' used in the study. Source indicates the original article from which the raw data were obtained. 99

Table 4-2: Summary statistics for Blomberg et al. 2003's K-statistic tests for phylogenetic signal in our dataset and the phylogenetic Analysis of Variance (phyloANOVA) tests for comparisons of various physiological variables between heterothermic and homeothermic bat species. M_b = body mass, T_b = normothermic body temperature, $T_b - T_{lc}$ = the measure of T_{lc} expressed as the difference between body temperature and lower critical temperature, T_{uc} = the upper critical temperature, T_b at T_{lc} = the calculated body temperature at the lower critical temperature, T_b at T_{uc} = the calculated body temperature at the upper critical temperature and ΔT_b = the difference in body temperature between the thermal limits. All spp. indicates that all 29 species were included, homeothermic spp. indicates that a subset of only homeothermic species were used ($n = 11$), and heterothermic spp. indicates that a subset of only heterothermic species were used ($n = 18$). * $p < 0.05$, ** $p \leq 0.01$, non-significant values are not reported. 103

Table 4-3: The calculated mean evaporative water loss values at the respective thermal limits for each species where data was available. 104

Acknowledgments

I thank my thesis advisor Professor Barry Gordon Lovegrove for his confidence in me and allowing me academic freedom to pursue my research interests. Your unconventional approach to supervising, the big-picture view that you have about science and life, and general DIY attitude has taught me more than I could express here. You have elevated my thinking and empowered me to teach others.

I thank Christopher Edgar Duigan for being a pillar of support and encouragement throughout my time spent in Pietermaritzburg and for introducing me to the theatrical side of life. You have been there to keep me grounded.

I also thank Barry, Chris and the poppets for their open home and treating me as family.

I thank my own family, named in the dedication, for their encouragement, support, love and belief in me.

I thank Professor Andrew Alek Tuen and the University of Malaysia Sarawak for hosting me during the time that I spent conducting fieldwork in Borneo.

I would also like to thank my colleague Danielle Levesque, with whom I spent much time in Borneo. Thank you for your pearls of wisdom, encouragement and friendship. Thank you also for your helping hand during field trips and laboratory experiments.

I thank Ebrahim Ally for all of his technical assistance and willingness to troubleshoot problems whether it be for the laboratory or during the period while I was teaching. Similarly, I thank Professor Colleen Downs for her support, encouragement and advice during the period while I was teaching.

I thank Andrew McKechnie for his valuable comments and useful discussions during various stages of my study. I thank Kwezi Mzilikazi and Diane Smith for their comments on my first chapter and thank Mark Brigham for his comments on my fourth chapter.

I thank Tunmise Otitoju, Miyn Naharuddin, Anthony Pine, Sally Soo, Cindy Peter, Matthew Jenang, Frances Dai Sze Hii, Luisa Setia, Cecilia Emang Ajeng, Matthew Jenang, Adi shabrani, Ben Karin, Yap Pui Kwan, Rahah Binti Mohamad Yakup and Mohamad Hasri Al-Hafiz Bin Haba, as well as the rest of my extended friend circle in Malaysia and staff at the IBEC office, who were always willing to lend a helping hand, and for showing me some of the wonders and varied cultures that Borneo has to offer.

I am grateful to the Bidayuh communities of the Barieng and Tanjong villages in Singai for access to their forest and use of their facilities. Similarly, I thank the friendly staff at Sama Jaya Nature Reserve for allowing me free access to the reserve.

I thank Dr. Samuel Kiyui and his staff at Country Veterinary Clinic for their exuberance and curiosity.

I thank my colleagues at Nelson Mandela University for agreeing to grant me leave of absence from work to write-up and complete my thesis.

Lastly, I would like to convey my gratitude to the GreenMatter Fellowship and their partners, the South African National Research Foundation, the Ministry of Higher Education of Malaysia and the Gay Langmuir Scholarship who provided funding for the project. Without all of your support, this study would not have been possible.

Chapter one – Southeast Asia: an evolutionarily insightful but rapidly deteriorating ecosystem

Shaun Welman^{1*}

¹ School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

*Corresponding author: welmans@ukzn.ac.za

Note: The manuscript style has been formatted in British English

Abstract

Despite the uncertainty regarding the divergence dates of crown Mammalia, placental mammals are thought to have evolved in warm, tropical conditions. Given that many of the islands in Southeast Asia have remained within the tropics since at least the mid-to-late Cretaceous, mammals that colonized these islands, before the onset of global cooling during the Cenozoic, are likely to have retained plesiomorphic traits through stabilizing selection. As such, physiological and ecological data collected from those mammalian lineages would provide key insight into the phenotype of the common ancestor of placental mammals. Unfortunately, many Southeast Asian mammals seem particularly vulnerable to the negative effects associated with habitat loss and global warming and the window of opportunity for data collection is quickly disappearing. Fortunately, studies that adopt a mechanistic approach could yield data that are vital for assessing the potential of Southeast Asian mammals to survive global warming and simultaneously document information that is evolutionarily insightful.

Recent insight into the evolution of placental mammals

Based upon character state reconstruction analyses, it has been proposed that the hypothetical common ancestor of placental mammals, colloquially named *Schreödinger*, was a small, insectivorous and nocturnal shrew-like mammal (O'Leary et al., 2013). *Schreödinger* is likely to have had a body temperature (T_b) of approximately 34°C (Lovegrove, 2012a; b) and to have had the capacity to employ adaptive heterothermy (Lovegrove, 2012c); which was taken to mean that it had the ability to enter torpor or hibernation. Notably, here and throughout the thesis, I have chosen to follow a similar definition of adaptive heterothermy. However, the use of heterothermy in this context does not obviate the presumption that *Schreödinger* likely also had a highly variable T_b ; not unlike that observed in phylogenetically closely related taxa such as monotremes and marsupials (reviewed by Gaughan et al., 2015), xenarthra (McNab, 1985; Boily, 2002; Toledo et al., 2017), and a few placental mammals such as tenrecs (Levesque and Lovegrove, 2014).

Confidence in the idea that Schröwdinger was a heterotherm is central to the understanding of how mammals could have persisted until now. Importantly, the physiological benefits associated with torpor and hibernation significantly improves survivability in extant species (Geiser and Turbill, 2009; Turbill et al., 2011), especially during adverse environmental conditions (Nowack et al., 2017). Based on this observation, it has been argued that mammals only survived the Cretaceous-Paleogene (K-Pg) extinction event at around 65.5 million years ago (mya) because of their capacity to endure the environmental turmoil following the meteorite impact at Chicxulub, Mexico, through the use of adaptive heterothermy (Lovegrove et al., 2014b). However, the general acceptance of such an argument is hindered by the highly contentious debate regarding the date of divergence for Mammalia and the number of mammalian groups that survived the K-Pg extinction (Bininda-Emonds et al., 2007; Meredith et al., 2011; O'Leary et al., 2013; Foley et al., 2016; Halliday et al., 2017).

Whereas some argue that the major radiation of placental mammals occurred shortly after the K-Pg boundary (i.e. < 65.5 Mya) (O'Leary et al., 2013; Halliday et al., 2017), others argue that all of the major groups of placental mammals were already present during the mid-to-late Cretaceous (ca. 90 – 65.5 mya) (Bininda-Emonds et al., 2007; Meredith et al., 2011; Foley et al., 2016). Consequently, we lack a definitive understanding of the environmental conditions under which placental mammals would have evolved. This uncertainty has had a profound influence on how mammalian traits are perceived to have evolved, and limits the context of the adaptations needed for their survival. Now, thanks to recent improvements in our understanding of the paleotopography during the Cretaceous Period, there has been a change in perception regarding the paleoclimate during that time, that could provide a better framework for understanding the evolution of placental mammals.

The latest thinking is that the Cretaceous Period was warmer and wetter than previously suggested (Hay, 2017), lending credence to the argument that tropical rainforest-like biomes already existed by the end of the Cretaceous (Couvreur and Baker, 2013). Thus, whereas earlier studies claimed that tropical rainforests evolved only after the K-Pg boundary during the Paleogene (ca. 65-62 mya: Morley, 2000; Jaramillo et al., 2010), paleoclimatic data now support Couvreur and Baker (2013)'s argument that it evolved much earlier, during the mid-Cretaceous (ca. 100 mya) (Fig. 1-1). If the latter is true, it would provide robust support for the idea that placental mammals evolved under warm, tropical conditions because irrespective of whether Schröwdinger evolved as early as 100 mya (Foley et al., 2016) or as late as 66 mya (O'Leary et al., 2013), placental mammals would still have evolved under tropical rainforest-like conditions and remained therein, for the most part, until the onset of global cooling during the Cenozoic (ca. 50 mya) (Fig. 1-1).

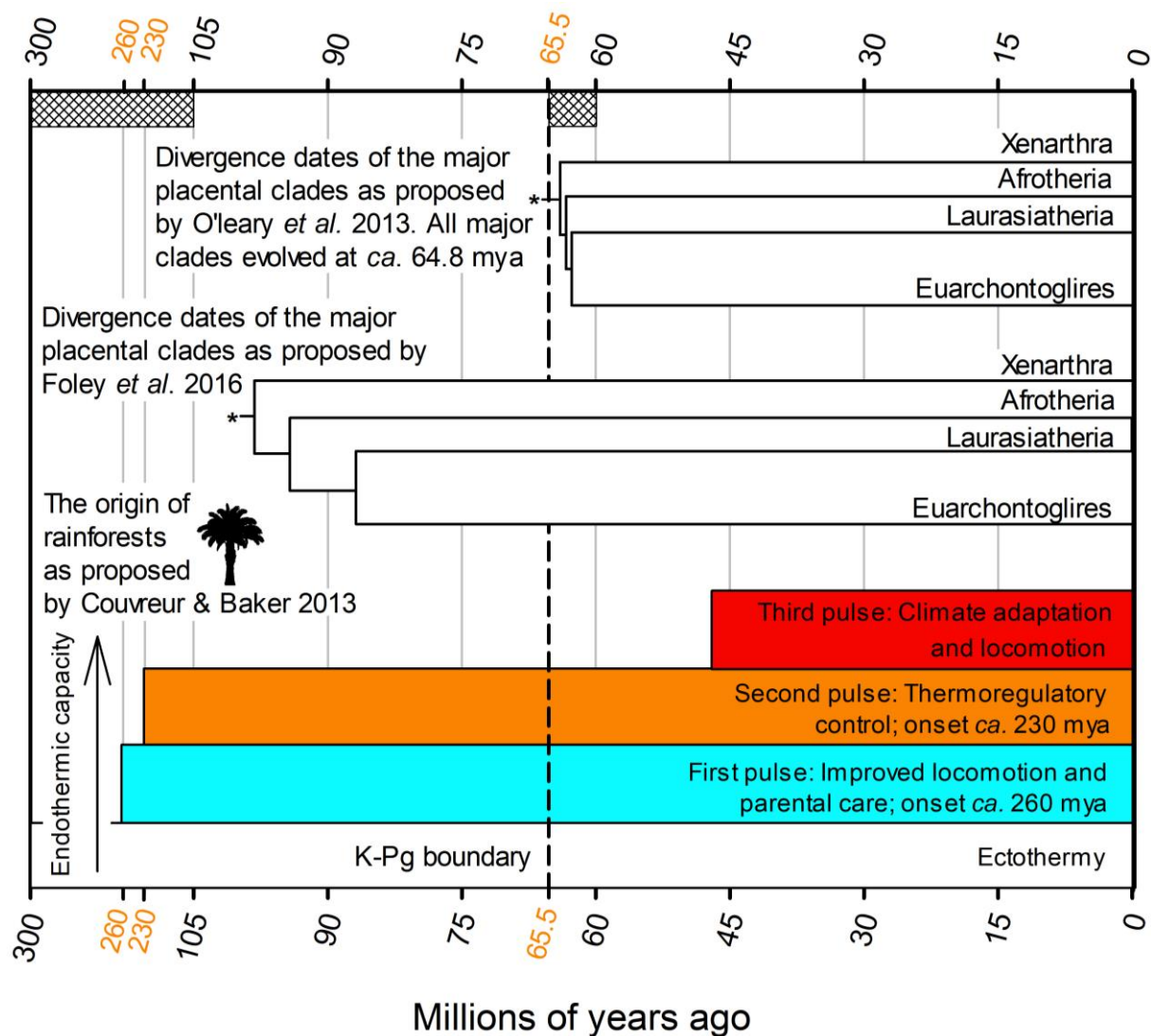


Figure 1-1: The overlap between the evolutionary history of endothermy in mammals according to Lovegrove (2017), the origin of the placental ancestor and the origin of rainforest environments. The respective date of divergence of the superordinal groups of placental mammals are depicted according to O'Leary et al. (2013) and Foley et al. (2016), and the proposed origin of rainforest environments is according to Couvreur and Baker (2013). The time periods under the cross-hatch shading are not to scale. The asterisk represents the proposed origin of the placental ancestor. The palm silhouette represents the proposed origin of rainforests at approximately 100 mya, and was obtained from silhouettesfree.com under a Creative Commons License. The dashed line represents the Cretaceous-Paleogene (K-Pg) boundary.

The potential for Southeast Asian mammals to provide meaningful insight into the placental ancestor

Mammals that colonised tropical islands during the Early Cenozoic, prior to the onset of global cooling, would, in general, have retained plesiomorphic traits through stabilising selection (Hansen, 1997; Lobban et al., 2014). The potential for stabilising selection certainly seems high within Southeast Asian mammals because based on the theory of continental drift (Wegener, 1912), a reconstruction of Earth's landmasses suggests that many of the Southeast Asian islands have remained within the tropics for at least the last 100 million years (Scotese, 1991). Thus, aside from some changes in floristic composition (Jablonski, 2003; Couvreur and Baker, 2013), the tropical environment in Southeast Asia would have remained moderately stable from the time that *Schreödinger* evolved, until now.

In combination with the notion that Southeast Asian mammals would have experienced stabilising selection that favoured the retention of plesiomorphic traits, there also are several species that are conveniently positioned phylogenetically to provide meaningful evolutionary insight into the ancestral phenotype. For instance, tarsiers (Tarsiidae), colugos (Cynocephalidae) and treeshrews (Tupaiaidae and Ptilocercidae) are key representatives of the orders Primates, Dermoptera and Scandentia respectively. Collectively, these three orders form the Euarchonta (Fig. 1-2). Whereas tarsiers are phylogenetically basal within one of the two suborders of primates, namely the Haplorrhini (tarsiers, monkeys, apes and humans) (Hartig et al., 2013), colugos are the only extant representatives of Dermoptera, the sister group to the primates. Furthermore, treeshrews are the only representatives of order Scandentia, the sister group to the Clade Primatomorpha (Dermoptera and Primates) (Roberts et al., 2011). As such, this unique combination of taxa has the potential to provide meaningful insight into the common ancestor of Euarchontoglires (Euarchonta and Glires), one of four superordinal clades in Placentalia (Foley et al., 2016) (Fig. 1-1 and 1-2). Consequently, understanding these species would significantly improve our understanding of the placental ancestor.

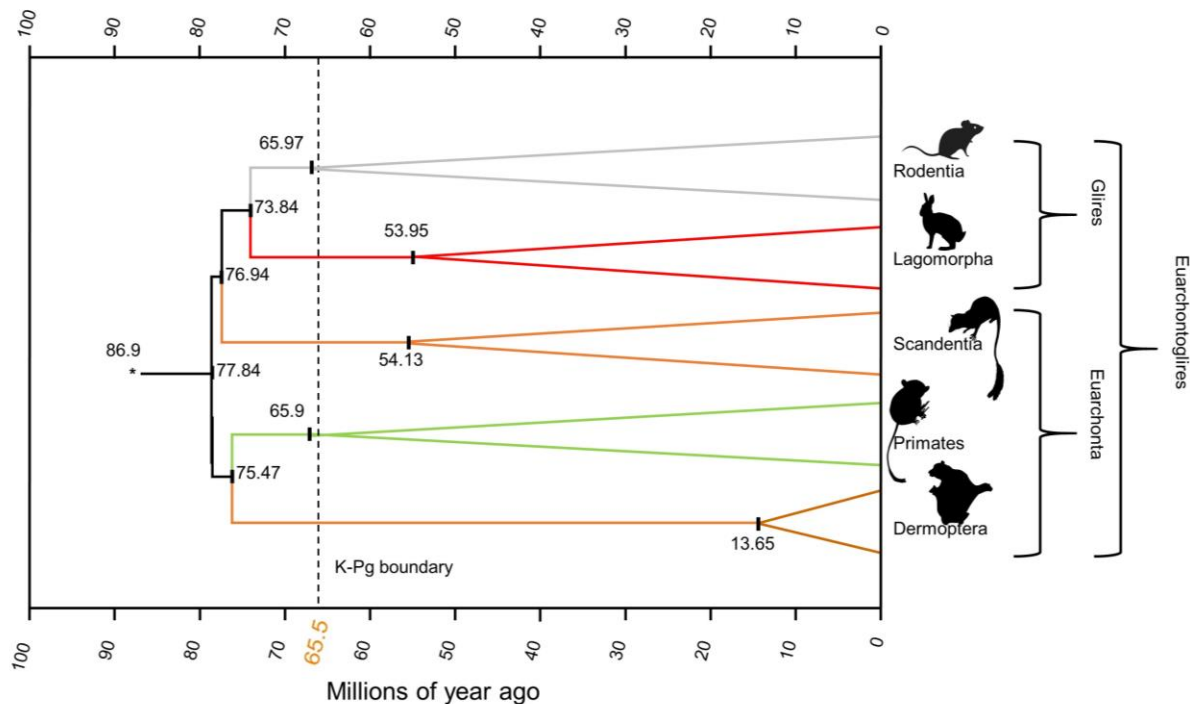


Figure 1-2: The phylogenetic relationship of the mammalian Orders within the clade Euarchontaglires. The date of divergence at each node is based on the long fuse model of Foley et al. (2016). The asterisk represents the date of divergence between the Euarchontoglires and Laurasiatheria superordinal groups. The mouse, rabbit, tree shrew, tarsier and flying lemur animal silhouettes depict commonly recognizable animals within their respective Order. Animal silhouettes were obtained from Phylopic.org under a Creative Commons License. The dashed line represents the Cretaceous-Paleogene (K-Pg) boundary.

The value of Southeast Asia as a model system to test evolutionary hypotheses regarding the evolution of endothermy in placental mammals

The ability to regulate T_b through endogenous heat production (i.e. endothermy) and remain active independently of ambient temperature (T_a) is thought to be the main reason why mammals became so successful after the demise of the non-avian dinosaurs (Lovegrove, 2012a). However, the evolutionary history that resulted in this ability is uncertain. Recently, it was claimed that mammals evolved higher T_b and improved homeothermy (i.e. the maintenance of T_b within a relatively narrow range) through an iterative increase in metabolic rate that progressively fuelled endothermy, and that this increase in metabolism was driven by three major pulses in endothermic capacity (Lovegrove, 2017) (Fig. 1-1). The basis of this claim was a comprehensive review and synthesis of previous hypotheses that have been

proposed to explain the evolution of endothermy in birds and mammals, as well as the available morphological evidence to support the arguments presented.

Briefly, Lovegrove (2017) claims that the first pulse in the evolution of endothermy was related to increases in metabolic capacity by stem synapsids, therapsids and cynodonts due to the activity demands and selection for enhanced embryonic development and post-hatching parental care associated with colonising land (*sensu* Bakker, 1971; Bennett and Ruben, 1979; Farmer, 2000; Koteja, 2000). It is also claimed that, due to the large body sizes of synapsids and early therapsids, these creatures had high thermal inertia, which resulted in a rudimentary capacity to achieve homeothermy and maintain a stable T_b (McNab, 1978). As a consequence, selection would have favoured bodily functions that became optimized within a narrower range of T_b (*sensu* Heinrich, 1977). The second pulse in the evolution of endothermy was then initiated by the extreme miniaturization in the body size of therapsids during the Late Triassic and Jurassic. It follows that as these therapsids shrunk, they would have progressively lost their capacity to retain body heat through thermal inertia. To maintain the benefits accrued during the period of large-body-size homeothermy, they would have needed to increase their thermoregulatory control (Lovegrove, 2017). This would have been achieved through a combination of enhanced endothermic capacity to elevate T_b (*sensu* McNab, 1978) and a reduced rate of heat loss through the evolution of fur (Lovegrove, 2017). Consequently, these improvements in thermoregulation would have allowed therapsids to invade and exploit a vacant nocturnal niche (Crompton et al., 1978). Thereafter, endothermy would have been advanced by the increases in metabolic rate associated with the evolution of larger brains and other neuronal accompaniments needed for a nocturnal existence (Rowe et al., 2011). Finally, the third pulse in the evolution of mammalian endothermy is claimed to be related to further increases in aerobic capacity by early mammals due to the evolution of terrestrial cursoriality and adaptations to the change in climate following the onset of global cooling during the early Cenozoic (Lovegrove, 2000; Lovegrove and Mowoe, 2014; Lovegrove, 2017). It is also argued that mammals could only have reinvaded a diurnal niche, following the demise of the non-avian dinosaurs, if they had a sufficiently large thermal gradient ($T_b - T_a$) that allowed passive heat transfer from the animal to the environment, minimising the need for evaporative cooling and reducing the risk of dehydration (Crompton et al., 1978). Though, the theoretical basis for the relationship between diurnality and endothermy is elusive. Could diurnality have been a driver for enhanced endothermy, a benefit of enhanced endothermy or simply reflect the absence of the previously dominate dinosaur taxa?

Given the long history of tropical conditions throughout many Southeast Asian islands and the potential of the continuously tropical conditions to have favoured the retention of plesiomorphic traits in Southeast Asian mammals, Southeast Asia is an ideal region to study

the evolution of endothermy in mammals. For instance, based on the triphasic iterative model for the evolution of endothermy discussed above, one would expect ancient taxa such as colugos and tarsiers, which have remained in tropical conditions since their respective date of divergence, to have a low T_b , a low endothermic capacity and to be heterothermic. Accordingly, they should have low metabolic rates and some propensity to enter torpor. However, no physiological data exist for colugos and the limited data that do exist for tarsiers provide evidence of a low metabolic rate and T_b (McNab and Wright, 1987; Lovegrove et al., 2014a), but not of adaptive heterothermy. Though, given the small number of studies, the current lack of evidence of adaptive heterothermy in tarsiers is not to say that adaptive heterothermy does not occur in this clade. Furthermore, indigenous Southeast Asian mammals, the ancestors of which have colonised islands in Southeast Asian before the onset of global cooling during the Cenozoic, would have experienced similar, warm environmental conditions. Hence, the influence of climate adaptation on endothermy, in response to cold, would presumably have been minor. As such, these Southeast Asian mammals provide an opportunity for a physiological comparison between diurnal and nocturnal, and arboreal and cursorial that could be useful to determine the respective influence of diurnality and enhanced locomotion on mammalian endothermy. In general, a better understanding of the physiology of Southeast Asian mammals would greatly improve our understanding of the evolutionary drivers that favoured the selection of homeothermy and higher T_b s. Yet, despite the possible evolutionary significance, physiological data for mammals from Southeast Asia are scarce (Lovegrove, 2000; Khaliq et al., 2015).

A race against time: The threat of habitat loss and global warming in the tropics

Tropical regions have the highest biodiversity on earth, usually boasting species-rich ecosystems (Rohde, 1992; den Tex and Leonard, 2013; Jablonski et al., 2013; Jansson et al., 2013). However, given that population density tends to decrease with an increase in species richness and that specious ecosystems also tend to host a higher number of rare species, tropical ecosystems are vulnerable to cascading extinctions and general ecosystem collapse (Kaneryd et al., 2012). Ensuring the integrity of these ecosystems is of paramount importance because, irrespective of any ethical obligation or academic interests (as previously discussed), they form an integral component of the hydrological cycle and general atmospheric circulation (Bruijnzeel, 2004; Warwick et al., 2013). The collapse of tropical ecosystems would thus have a globally significant bearing on air quality and precipitation (Gedney and Valdes, 2000; Werth and Avissar, 2002; Laurance, 2004; Pyle et al., 2011). Yet, despite their importance, tropical regions are globally under severe pressure from anthropogenic-related effects.

Land use change has been extensive throughout the tropics and is currently the dominant threat to tropical ecosystems (Geist and Lambin, 2002; Achard et al., 2014); especially within Southeast Asia (Reynolds et al., 2011; Gaveau et al., 2016; Miettinen et al., 2016). Repurposing natural land has widespread detrimental implications for biodiversity and ecosystem integrity due to the combination of direct effects and a cascade of indirect effects (Martínez-Ramos et al., 2016; Barnes et al., 2017). The obvious direct effect of land use change is the eradication of species and the reduction in the total area available for species to inhabit. However, in addition to the overall decline in available habitat, land use change increases fragmentation of the remaining habitat. This promotes edge effects and also affects the microclimate within these habitats, making them less hospitable for many species (Turner and Corlett, 1996; Laurance, 2004; Tuff et al., 2016). Thus, land use change could indirectly facilitate changes in species communities (Martínez-Ramos et al., 2016; Barnes et al., 2017). For example, habitat fragmentation decreases the total canopy cover in forests and allows more light to reach the understory. The increase in light may favour certain plant species and lead to their proliferation, at the expense of other species (Luken et al., 1997). Consequently, structural changes to the plant community may promote changes to the faunal community (Martínez-Ramos et al., 2016), that, in turn, would perpetuate ecosystem changes.

Tropical species are also threatened by the current, anthropogenic-related, global warming. It is generally accepted that species will respond to global warming through a compensatory migration poleward or towards higher altitude (Walther et al., 2002; Root et al., 2003; Parmesan, 2006). However, globally, many species may be trapped in isolated habitat fragments due to land use change (Hansen et al., 2001). Furthermore, many tropical species live on islands and have limited dispersal potential. At some point, these species will no longer be able to move and will be forced to endure or adapt to their new conditions or become extinct. In this regard, studying their physiology provides a useful mechanistic link between environmental variability, individual tolerances and performance, and thus the species' potential to persist in the face of a warming climate (Ricklefs and Wikelski, 2002; Sherwood and Huber, 2010; Lovegrove et al., 2014a). In the case of Southeast Asia, such data collection has the secondary benefit of documenting information that is potentially vital for understanding the evolution of mammals. Unfortunately, the physiological data required to assess species tolerances are often unavailable for the tropics (Lovegrove, 2000; Huey et al., 2012). Though, available data suggests that many tropical and semi-tropical mammals have comparatively low ($<36^{\circ}\text{C}$) and thermolabile T_b (Racey and Stephenson, 1996; Lovegrove and Génin, 2008; Stawski and Geiser, 2012; Lovegrove et al., 2014a).

Temperature has a biphasic effect on biological processes. Initially there is a positive relationship as the rate of processes increase with temperature, up to a maximum point.

Thereafter, hyperthermia ensues as further increases in temperature results in a rapid decline in the rate of said processes (Boyles et al., 2011; Tattersall et al., 2012). Endotherms can experience varying degrees of hyperthermia ranging from non-lethal reversible effects to death, depending on the rate of heat storage, the absolute increase in their T_b , as well as the duration that they experience such elevated T_b s (Schmidt-Nielsen, 1997). For mammals, the mean lethal T_b is seemingly ca. 43°C (Prothero, 2015). Thus, one view could be that mammals with lower T_b can accommodate more heat storage before reaching the lethal threshold. However, it is also reported that, in general, endotherms (birds and mammals) seemingly only survive increases in T_b of up to 6°C relative to their normothermic T_b (Schmidt-Nielsen, 1997). At the outset, these two views seem contradictory. However, the normothermic T_b of species used to calculate the mean lethal T_b of 43°C for mammals were predominantly $\geq 37^\circ\text{C}$, which thus lends credence to the argument that endotherms, at least in the case of mammals, only survive up to 6°C increases in T_b relative to their normal T_b . Furthermore, the optimal temperature and effective range of temperatures that biological process function seems to vary between processes and species (Schmidt-Nielsen, 1997; Boyles et al., 2011; Tattersall et al., 2012). Thus, considering this biphasic effect of temperature on biological processes, together with the hypothesis that natural selection would have favoured the optimisation of bodily functions at or close to the species-specific T_b (McNab, 1978), it seems more likely that species with lower T_b risk deleterious effects at lower absolute T_b s relative to their higher T_b counterparts.

Based on the view that the level of hyperthermia perceived by endotherms is likely to be T_b dependent, the low T_b of many tropical and semi-tropical mammals is particularly concerning. As a consequence of their low T_b , they encounter relatively small temperature differentials between themselves and their environment. The smaller thermal gradient (i.e. low $\Delta T = T_b - T_a$) compromises their ability to passively dissipate excess body heat or maintain T_b within species-specific ranges because heat dissipation via conduction and radiation diminishes as ΔT approaches zero (Schmidt-Nielsen, 1997; Sherwood and Huber, 2010). Once $T_a \geq T_b$ evaporative cooling becomes the only means of dissipating heat from the body (McKechnie and Wolf, 2004; Cory Toussaint and McKechnie, 2012; Withers et al., 2012) but it requires at least a 1-2°C differential between the animal's skin and the wet-bulb temperature (Sherwood and Huber, 2010). Thus, whereas mammals inhabiting higher latitudes usually encounter large water vapour pressure deficits that favour evaporative cooling (Walsberg, 2000), the high relative humidity conditions in the tropics could potentially retard evaporative cooling and lead to fatal levels of heat storage (Mora et al., 2017), even in seemingly mobile species such as bats (Welbergen et al., 2008). As such, many tropical mammals could already be living close to the upper limit of their thermotolerance and are at high risk of severe

hyperthermia due to the predicted increase in global temperatures (Lovegrove et al., 2014a). Crucially, the aggressive land use change in Southeast Asia could exacerbate the effects of global warming because as previously mentioned, habitat fragmentation would diminish the potential of forests to buffer environmental variability and would increase the exposure of many species to potentially devastating environmental stochasticity (Hansen et al., 2001).

Conclusion

Tropical mammals, especially South East Asian species, have the potential to provide key insight into evolutionary processes in mammals. However, the availability of natural ecosystems and the time remaining within which to study them is quickly disappearing. Southeast Asia is under severe threat due to aggressive land use change and the effects of global warming. It is imperative that steps are taken to improve conservation efforts to prevent wide-spread ecosystem collapse. In this regard, studies that adopt a mechanistic approach to studying the physiological effects of global warming on tropical mammals would be strategic because they could lead to a better understanding of the fate of tropical mammals, greatly assist conservation planning and yield potentially significant evolutionary insight.

Problem statement and Thesis rationale

The tropics are highly speciose, make a significant contribution to the global primary productivity, are an integral component of the global atmospheric circulation and, for evolutionary biologists, potentially provide key insights into the evolution of placental mammals (Rohde, 1992; Malhi, 2012; den Tex and Leonard, 2013; Jablonski et al., 2013; Jansson et al., 2013). However, there is a vast gap in our understanding of the future of this region. The widely accepted range shifts that species are expected to undertake in response to climate change would suggest that many insular species, with limited dispersal capacity, face extinction. Importantly, the view that species will migrate towards the poles or towards higher altitude (Walther et al., 2002; Root et al., 2003; Parmesan, 2006) is based on species distribution models (SDMs; synonymous with climate-envelope models, niche models or bioclimatic models), which do not adequately consider all dynamics involved. These models are rooted in the “ecological niche theory” (Hutchinson, 1957) and rely on the principle of niche conservatism, that is to say that species’ will retain ancestral ecological characteristics and seek to remain within similar niches (Wiens and Graham, 2005). However, even though SDMs have been considered to be valuable conservation tools because some of them have successfully predicted the distributional patterns of certain species (Pearson and Dawson, 2003; Araújo et al., 2004; Araújo et al., 2011; Struebig et al., 2015), they rely upon several unrealistic assumptions, and are thus fundamentally flawed.

The most significant assumptions that SDMs make are i) that the current distribution of species and the associated large-scale environmental conditions accurately reflect the species *preferred* niche (Guisan and Thuiller, 2005; Wiens et al., 2009), ii) that species can migrate uninhibited across heterogeneous and often highly transformed landscapes to reach suitable areas, at a rate similar to that of climate change (Pearson and Dawson, 2003; Wiens et al., 2009), iii) that species will respond independently of others and thus SDMs essentially disregard ecological effects (Davis et al., 1998; McCarty, 2001), and iv) that species will not be able to tolerate or adapt to future conditions (Chevin et al., 2010; Chown et al., 2010; Fuller et al., 2010; Boyles et al., 2011). Any assessment of a species vulnerability to global warming should be multifaceted, especially large-scale models that aim also to predict future species responses. These models would thus require a framework of understanding of the species ecology, physiological sensitivity, risk of exposure, resilience, as well as adaptability due to genetic variability and phenotypic plasticity. Exploring and understanding the mechanistic relationship between climate change and the physiological responses of animals to environment variability will provide a much needed subset of the framework for future large-scale models (Williams et al., 2008; Kearney et al., 2010; Huey et al., 2012).

To date, most of the physiological research related to global warming is biased towards ectotherms and focus on the temperate zone (Dillon et al., 2010; Lovegrove et al., 2014a). Less than 1% of the long-term data stems from the tropics (Rosenzweig et al., 2008). Nevertheless, thermotolerance is a quick and convenient measure that could be used to assess species vulnerability to global warming because it represents a rigid physiological limit to what animals may endure, and thus provides useful insights to species adaptability to global warming (Sherwood and Huber, 2010). Heat stress is dependent on a species physiological susceptibility and exposure (Ruddell et al., 2010). As such, small arboreal tropical mammals seem particularly at risk of hyperthermia because they store heat quickly due to their low thermal inertia. Moreover, their exposed lifestyles make them susceptible to environmental perturbations and the small thermal gradient reduces their capacity to passively dissipate excess body heat (Sherwood and Huber, 2010; Tattersall et al., 2012; Lovegrove et al., 2014a). Thus, gathering information about species thermoregulatory capacity, coupled with their habits and available microclimates, should be a priority. This thesis was aimed at addressing some of these gaps in our knowledge.

Research objectives, study species and approach

The main objective of this study was to evaluate the vulnerability of tropical, arboreal small mammals to heat stress. In addition, the data collected provide further insights into the evolution of endothermy in mammals. I used an integrated field and laboratory design to

investigate the thermoregulatory capacity of two small nocturnal arboreal tropical endotherms namely western tarsiers (*Cephalopachus bancanus*) and the lesser dog-faced fruit bat (*Cynopterus brachyotis*) from the island of Borneo. It is predicted that by the end of this century, Borneo would have experienced a mean T_a increase of 2.6 °C relative to the temperatures during 1961-1990 (Numerikal, 2009). However, even though this increase may be lower than the predicted average global increase, it is expected that Borneo will experience several pulses of record high temperatures during 2028, 2048, 2061 and 2079, with spikes in T_a of 1.9 - 2.7°C, 2.2 - 2.8°C, 2.9 - 3.4°C and 3.9 - 4.3°C respectively (Numerikal, 2009). Given the catastrophic die-offs observed during anomalous heat waves in other tropical species (Welbergen et al., 2008), these spikes could have devastating effects.

Tarsiers are small (80 – 150g; but see Clarke, 1943) primates that inhabit the forests of South-east Asia (MacKinnon and MacKinnon, 1980; Crompton and Andau, 1986; 1987; Neri-Arboleda et al., 2002; Groves and Shekelle, 2010). As previously mentioned, they are the sister group of the Anthropoids and basal in Haplorrhini (Hartig et al., 2013). Tarsiers evolved during the Eocene and have remained within the tropics ever since (Jablonski, 2003; Simons, 2003). Thus, given their habitat stability, and the close phylogenetic relationship of tarsiers to the Strepsirrhini, wherein adaptive heterothermy is common (Dausmann, 2014; Dausmann and Warnecke, 2016), tarsiers can provide valuable insights into the evolution of endothermy. Although some ecological (Jablonski and Crompton, 1994; Gursky, 2000; Dagosto et al., 2001; Gursky, 2002; Crompton et al., 2010) and genetic (Schmitz et al., 2001; Hartig et al., 2013) data exist for tarsiers, physiological data are scant. Available physiological data exist for the Philippine tarsier (*Tarsius syrichta*) which have low T_{bs} (33.3°C - 33.8°C; McNab and Wright, 1987; Lovegrove et al., 2014a) and display no adaptive heterothermy. Based on these low T_{bs} , and hence compromised heat dissipation capacity due to a low ΔT , tarsiers are theoretically vulnerable to heat stress.

Lesser dog-faced fruit bats (*Cynopterus brachyotis*) are small (37g) foliage-roosting generalist frugivores that are widely distributed throughout Asia (Liat, 1970; McNab, 1989; Abdullah, 2003). Relative to other bats, *C. brachyotis* have a high T_b (ca. 36.5°C; McNab, 1989). Thus, they should be less vulnerable to heat stress because, theoretically, they would be able to maintain a favourable thermal gradient for longer than lower T_b counterparts. In addition, *C. brachyotis* are heterotherms (McNab, 1989). Using torpor would certainly make them more resilient to indirect effects of global warming (Geiser and Turbill, 2009; Geiser et al., 2011; Geiser and Brigham, 2012), but recent work argues that torpor could potentially make them more resilient to direct effects as well (Bondarenko et al., 2014). Further, generalist species are expected to be less affected by climate change (Gilman et al., 2010; Hof et al., 2012; Gough et al., 2015; Afonso Silva et al., 2017) and are more likely to survive and persist

within their current habitats. Because these bats are important seed dispersers that also participate in pollination (Liat, 1970; Boon and Corlett, 1989; Tan et al., 1998), they have a significant contribution in maintaining ecosystem integrity. Thus, I wanted to confirm earlier thermoregulatory data as well as to assess whether there were any indications of adaptive heterothermy being beneficial at high T_a .

Quantifying all aspects of heat exchange between an endotherm and its environment, especially within its natural conditions, is difficult. However, the rate at which endotherms store heat may be modelled mathematically by rearranging the simplified heat balance equation of Schmidt-Nielsen (1997):

$$T_S = H_{MR} \pm H_C \pm H_R \pm H_E \quad \text{Equation 1}$$

where T_S is heat storage, H_{MR} is heat production from metabolic processes (always positive), H_C is the net heat exchange from the combined effects of conductive and convective heat transfer, H_R is the net heat exchange from radiation, and H_E the net heat exchange from evaporation (typically negative). Thus, to better understand the risk of heat stress in the study species, I focussed on the interaction between T_a , T_b , metabolism and evaporative heat loss. Furthermore, as an attempt to attain a more realistic understanding of metabolic and energetic implications of global warming for the study species, I related their physiological data to the microclimate (T_a and relative humidity) measured within their respective habitats. Another approach could have been to use physical models to quantify the heat exchange dynamics between the study animals and their respective environments. Typically, such an approach is used to determine operative temperatures and standard operative temperatures (Dzialowski, 2005), whereby operative temperature is an approximation of the net heat exchange via conduction, convection and thermal radiation between the animal and its environment without the influence of metabolic heat production or evaporative cooling, and where standard operative temperature is a measure of the rate of metabolic heat production required to compensate for the rate of heat lost observed for a set of standardized simulated environmental conditions. However, this approach was avoided based on the following considerations. Firstly, to be effective, these physical models necessarily must closely resemble the thermal properties of each target species, yet they are not able to account for non-metabolic thermoregulatory responses such as vasodilation and postural changes, that also influence the rate of heat exchange. Secondly, even though using physical models may have yielded data that are slightly more ecologically relevant, the specificity of such models limits the wider applicability of the data. Thus, given the urgency with which we need to improve our understanding of the likely responses of species to global warming, the use of T_a , although not perfect, was favoured because of the convenience of obtaining data, and the

general applicability of the data for understanding the physiological consequences of temperature across taxa.

I also sought to combine the data collected for *C. brachyotis* with thermoregulatory data available for other bat species to find corroborating physiological evidence to support claims that heterotherms can enter torpor at T_a typically though to be within or above thermoneutrality. Furthermore, based on the principles of heat exchange (equation 1), I developed *a priori* models that could also test if metabolic down-regulation at these high T_a had the potential to retard the rate of heat storage and minimize the use of evaporative water loss.

Literature cited

- Abdullah, M.T. (2003). *Biogeography and variation of Cynopterus brachyotis in Southeast Asia*. Doctorate PhD thesis, University of Queensland.
- Achard, F., Beuchle, R., Mayaux, P., Stibig, H.-J., Bodart, C., Brink, A., et al. (2014). Determination of tropical deforestation rates and related carbon losses from 1990 to 2010. *Glob. Change Biol.* 20(8), 2540-2554. doi: 10.1111/gcb.12605.
- Afonso Silva, A.C., Bragg, J.G., Potter, S., Fernandes, C., Coelho, M.M., and Moritz, C. (2017). Tropical specialist vs. climate generalist: diversification and demographic history of sister species of *Carlia* skinks from northwestern Australia. *Mol. Ecol.* 26(15), 4045-4058. doi: 10.1111/mec.14185.
- Araújo, M.B., Alagador, D., Cabeza, M., Nogués-Bravo, D., and Thuiller, W. (2011). Climate change threatens European conservation areas. *Ecol. Lett.* 14(5), 484-492. doi: 10.1111/j.1461-0248.2011.01610.x.
- Araújo, M.B., Cabeza, M., Thuiller, W., Hannah, L., and Williams, P.H. (2004). Would climate change drive species out of reserves? An assessment of existing reserve-selection methods. *Glob. Change Biol.* 10(9), 1618-1626. doi: 10.1111/j.1365-2486.2004.00828.x.
- Bakker, R.T. (1971). Dinosaur physiology and the origins of mammals. *Evolution* 25(4), 636-658.
- Barnes, A.D., Allen, K., Kreft, H., Corre, M.D., Jochum, M., Veldkamp, E., et al. (2017). Direct and cascading impacts of tropical land-use change on multi-trophic biodiversity. *Nat. Ecol. Evol.* 1(10), 1511-1519. doi: 10.1038/s41559-017-0275-7.
- Bennett, A.F., and Ruben, J.A. (1979). Endothermy and activity in vertebrates. *Science* 206(4419), 649-654. doi: 10.1126/science.493968.

- Bininda-Emonds, O.R.P., Cardillo, M., Jones, K.E., MacPhee, R.D.E., Beck, R.M.D., Grenyer, R., et al. (2007). The delayed rise of present-day mammals. *Nature* 446(7135), 507-512. doi: http://www.nature.com/nature/journal/v446/n7135/supinfo/nature05634_S1.html.
- Boily, P. (2002). Individual variation in metabolic traits of wild nine-banded armadillos (*Dasypus novemcinctus*), and the aerobic capacity model for the evolution of endothermy. *J Exp Biol* 205(20), 3207-3214.
- Bondarenko, A., Körtner, G., and Geiser, F. (2014). Hot bats: extreme thermal tolerance in a desert heat wave. *Naturwissenschaften* 101(8), 679-685. doi: 10.1007/s00114-014-1202-2.
- Boon, P.P., and Corlett, R.T. (1989). Seed dispersal by the lesser short-nosed fruit bat (*Cynopterus brachyotis*, Pteropodidae, Megachiroptera). *Malayan Nat. J.* 42, 251-256.
- Boyles, J.G., Seebacher, F., Smit, B., and McKechnie, A.E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* 51, 676-690.
- Bruijnzeel, L.A. (2004). Hydrological functions of tropical forests: not seeing the soil for the trees? *Agr. Ecosyst. Environ.* 104(1), 185-228. doi: <https://doi.org/10.1016/j.agee.2004.01.015>.
- Chevin, L.M., Lande, R., and Mace, G.M. (2010). Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLOS Biol.* 8(4), e1000357. doi: 10.1371/journal.pbio.1000357.
- Chown, S.L., Hoffmann, A.A., Kristensen, T.N., Angilletta, M.J., Stenseth, N.C., and Pertoldi, C. (2010). Adapting to climate change: A perspective from evolutionary physiology. *Clim. Res.* 43(1-2), 3-15. doi: 10.3354/cr00879.
- Clarke, R.W. (1943). The respiratory exchange of *Tarsius spectrum*. *J. Mammal.* 24(1), 94-96. doi: 10.2307/1374785.
- Cory Toussaint, D., and McKechnie, A.E. (2012). Interspecific variation in thermoregulation among three sympatric bats inhabiting a hot, semi-arid environment. *J. Comp. Physiol. B.* 182(8), 1129-1140. doi: 10.1007/s00360-012-0683-6.
- Couvreux, T.L., and Baker, W.J. (2013). Tropical rain forest evolution: palms as a model group. *BMC Biol.* 11(1), 48. doi: 10.1186/1741-7007-11-48.
- Crompton, A.W., Taylor, C.R., and Jagger, J.A. (1978). Evolution of homeothermy in mammals. *Nature* 272, 333-336.

- Crompton, R.H., and Andau, P.M. (1986). Locomotion and habitat utilization in free-ranging *Tarsius bancanus*: a preliminary report. *Primates* 27(3), 337-355. doi: 10.1007/BF02382075.
- Crompton, R.H., and Andau, P.M. (1987). Ranging, activity rhythms, and sociality in free-ranging *Tarsius bancanus*: a preliminary report. *Int. J. Primatol.* 8(1), 43-71. doi: 10.1007/BF02737113.
- Crompton, R.H., Blanchard, M.L., Coward, S., Alexander, R.M., and Thorpe, S.K. (2010). Vertical clinging and leaping revisited: locomotion and habitat use in the western tarsier, *Tarsius bancanus* explored via loglinear modeling. *Int. J. Primatol.* 31(6), 958-979. doi: 10.1007/s10764-010-9420-8.
- Dagosto, M., Gebo, D.L., and Dolino, C. (2001). Positional behavior and social organization of the philippine tarsier (*Tarsius syrichta*). *Primates* 42(3), 233-243. doi: 10.1007/BF02629639.
- Dausmann, K.H. (2014). Flexible patterns in energy savings: heterothermy in primates. *J. Zool.* 292(2), 101-111. doi: 10.1111/jzo.12104.
- Dausmann, K.H., and Warnecke, L. (2016). Primate torpor expression: ghost of the climatic past. *Physiology* 31(6), 398-408. doi: 10.1152/physiol.00050.2015.
- Davis, A.J., Lawton, J.H., Shorrocks, B., and Jenkinson, L.S. (1998). Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. *J. Anim. Ecol.* 67(4), 600-612.
- den Tex, R.J., and Leonard, J.A. (2013). A molecular phylogeny of Asian barbets: speciation and extinction in the tropics. *Mol. Phylogenet. Evol.* 68(1), 1-13. doi: 10.1016/j.ympev.2013.03.004.
- Dillon, M.E., Wang, G., and Huey, R.B. (2010). Global metabolic impacts of recent climate warming. *Nature* 467(7316), 704-706. doi: 10.1038/nature09407.
- Dzialowski, E.M. (2005). Use of operative temperature and standard operative temperature models in thermal biology. *J. Therm. Biol.* 30(4), 317-334. doi: <https://doi.org/10.1016/j.jtherbio.2005.01.005>.
- Farmer, C.G. (2000). Parental care: the key to understanding endothermy and other convergent features in birds and mammals. *Am. Nat.* 155, 326-334. doi: 10.1086/303323.

- Foley, N.M., Springer, M.S., and Teeling, E.C. (2016). Mammal madness: is the mammal tree of life not yet resolved? *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 371(1699). doi: 10.1098/rstb.2015.0140.
- Fuller, A., Dawson, T., Helmuth, B., Hetem, R.S., Mitchell, D., and Maloney, S.K. (2010). Physiological mechanisms in coping with climate change. *Physiol. Biochem. Zool.* 83(5), 713-720. doi: 10.1086/652242.
- Gaughan, J.B., Hogan, L.A., and Wallage, A. (2015). "Thermoregulation in marsupials and monotremes," in *Marsupials and monotremes: nature's enigmatic mammals*, eds. A. Klieve, L. Hogan, S. Johnston & P. Murray. (New York: Nova Science Publishers), 85-144.
- Gaveau, D.L.A., Sheil, D., Husnayaen, Salim, M.A., Arjasakusuma, S., Ancrenaz, M., et al. (2016). Rapid conversions and avoided deforestation: examining four decades of industrial plantation expansion in Borneo. *Sci. Rep.* 6, 32017. doi: 10.1038/srep32017 <https://www.nature.com/articles/srep32017#supplementary-information>.
- Gedney, N., and Valdes, P.J. (2000). The effect of Amazonian deforestation on the northern hemisphere circulation and climate. *Geophys. Res. Lett.* 27(19), 3053-3056. doi: 10.1029/2000GL011794.
- Geiser, F., and Brigham, R.M. (2012). "The other functions of torpor," in *Living in a seasonal world: thermoregulatory and metabolic adaptations*, eds. T. Ruf, C. Bieber, W. Arnold & E. Millesi. (Heidelberg, Berlin: Springer), 109-121.
- Geiser, F., Stawski, C., Bondarenko, A., and Pavey, C. (2011). Torpor and activity in a free-ranging tropical bat: implications for the distribution and conservation of mammals? *Naturwissenschaften* 98(5), 447-452. doi: 10.1007/s00114-011-0779-y.
- Geiser, F., and Turbill, C. (2009). Hibernation and daily torpor minimize mammalian extinctions. *Naturwissenschaften* 96(10), 1235-1240. doi: 10.1007/s00114-009-0583-0.
- Geist, H.J., and Lambin, E.F. (2002). Proximate causes and underlying driving forces of tropical deforestation: tropical forests are disappearing as the result of many pressures, both local and regional, acting in various combinations in different geographical locations. *BioScience* 52(2), 143-150.
- Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W., and Holt, R.D. (2010). A framework for community interactions under climate change. *Trends Ecol. Evol.* 25(6), 325-331. doi: 10.1016/j.tree.2010.03.002.

- Gough, L.A., Sverdrup-Thygeson, A., Milberg, P., Pilskog, H.E., Jansson, N., Jonsell, M., et al. (2015). Specialists in ancient trees are more affected by climate than generalists. *Ecol. Evol.* 5(23), 5632-5641. doi: 10.1002/ece3.1799.
- Groves, C., and Shekelle, M. (2010). The genera and species of Tarsiidae. *Int. J. Primatol.* 31(6), 1071-1082. doi: 10.1007/s10764-010-9443-1.
- Guisan, A., and Thuiller, W. (2005). Predicting species distribution: offering more than simple habitat models. *Ecol. Lett.* 8(9), 993-1009. doi: 10.1111/j.1461-0248.2005.00792.x.
- Gursky, S. (2000). Sociality in the spectral tarsier, *Tarsius spectrum*. *Am. J. Primatol.* 51(1), 89-101. doi: 10.1002/(SICI)1098-2345(200005)51:1<89::AID-AJP7>3.0.CO;2-7.
- Gursky, S. (2002). The behavioral ecology of the spectral tarsier, *Tarsius spectrum*. *Evol. Anthropol.* 11(6), 226-234. doi: 10.1002/evan.10035.
- Halliday, T.J.D., Upchurch, P., and Goswami, A. (2017). Resolving the relationships of Paleocene placental mammals. *Biol. Rev.* 92(1), 521-550. doi: 10.1111/brv.12242.
- Hansen, A.J., Neilson, R.R., Dale, V.H., Flather, C.H., Iverson, L.R., Currie, D.J., et al. (2001). Global change in forests: Responses of species, communities, and biomes. *Bioscience* 51(9), 765-779. doi: 10.1641/0006-3568(2001)051[0765:gcifro]2.0.co;2.
- Hansen, T.F. (1997). Stabilizing selection and the comparative analysis of adaptation. *Evolution* 51(5), 1341-1351. doi: 10.2307/2411186.
- Hartig, G., Churakov, G., Warren, W.C., Brosius, J., Makalowski, W., and Schmitz, J. (2013). Retrophylogenomics place tarsiers on the evolutionary branch of anthropoids. *Sci. Rep.* 3, 1756. doi: 10.1038/srep01756.
- Hay, W.W. (2017). Toward understanding Cretaceous climate—An updated review. *Sci. China Earth Sci.* 60(1), 5-19. doi: 10.1007/s11430-016-0095-9.
- Heinrich, B. (1977). Why have some animals evolved to regulate a high body temperature? *Am. Nat.* 111, 623-640.
- Hof, A.R., Jansson, R., and Nilsson, C. (2012). Future climate change will favour non-specialist mammals in the (sub)arctics. *PLOS ONE* 7(12), e52574. doi: 10.1371/journal.pone.0052574.
- Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A., Jess, M., and Williams, S.E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 367(1596), 1665-1679. doi: 10.1098/rstb.2012.0005.

- Hutchinson, G.E. (1957). Concluding remarks. *Cold Spring Harb. Symp. Quant. Biol.* 22, 415-457.
- Jablonski, D., Belanger, C.L., Berke, S.K., Huang, S., Krug, A.Z., Roy, K., et al. (2013). Out of the tropics, but how? Fossils, bridge species, and thermal ranges in the dynamics of the marine latitudinal diversity gradient. *Proc. Natl. Acad. Sci. U. S. A.* 110(26), 10487-10494. doi: 10.1073/pnas.1308997110.
- Jablonski, N.G. (2003). "The evolution of the Tarsiid niche," in *Tarsiers: past, present, and future*, eds. P.C. Wright, E.L. Simons & S. Gursky. (New Brunswick: Rutgers University Press), 35-49.
- Jablonski, N.G., and Crompton, R.H. (1994). Feeding-behavior, mastication, and tooth wear in the Western tarsier (*Tarsius bancanus*). *Int. J. Primatol.* 15(1), 29-59. doi: 10.1007/bf02735233.
- Jansson, R., Rodríguez-Castañeda, G., and Harding, L.E. (2013). What can multiple phylogenies say about the latitudinal diversity gradient? a new look at the tropical conservatism, out of the tropics, and diversification rate hypotheses. *Evolution* 67(6), 1741-1755. doi: 10.1111/evo.12089.
- Jaramillo, C., Hoorn, C., Silva, S.A., Leite, F., Herrera, F., Quiroz, L., et al. (2010). "The origin of the modern Amazon rainforest: implications of the palynological and palaeobotanical record," in *Amazonia, Landscape and Species Evolution: A look into the past*, eds. C. Hoorn & F.P. Wesselingh. (Oxford: Blackwell Publishing), 317-334.
- Kaneryd, L., Borrvall, C., Berg, S., Curtsdotter, A., Eklöf, A., Hauzy, C., et al. (2012). Species-rich ecosystems are vulnerable to cascading extinctions in an increasingly variable world. *Ecol. Evol.* 2(4), 858-874. doi: 10.1002/ece3.218.
- Kearney, M., Simpson, S.J., Raubenheimer, D., and Helmuth, B. (2010). Modelling the ecological niche from functional traits. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 365(1557), 3469-3483. doi: 10.1098/rstb.2010.0034.
- Khalik, I., Fritz, S.A., Prinzing, R., Pfenninger, M., Böhning-Gaese, K., and Hof, C. (2015). Global variation in thermal physiology of birds and mammals: evidence for phylogenetic niche conservatism only in the tropics. *J. Biogeogr.* 42(11), 2187-2196. doi: 10.1111/jbi.12573.
- Koteja, P. (2000). Energy assimilation, parental care and the evolution of endothermy. *Proc. R. Soc. B.* 267, 479-484. doi: 10.1098/rspb.2000.1025.

- Laurance, W.F. (2004). Forest-climate interactions in fragmented tropical landscapes. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 359(1443), 345-352. doi: 10.1098/rstb.2003.1430.
- Levesque, D.L., and Lovegrove, B.G. (2014). Increased homeothermy during reproduction in a basal placental mammal. doi: 10.1242/jeb.098848.
- Liat, L.B. (1970). Food habits and breeding cycle of the Malaysian fruit-eating bat, *Cynopterus brachyotis*. *J. Mammal.* 51(1), 174-177. doi: 10.2307/1378555.
- Lobban, K.D., Lovegrove, B.G., and Rakotondravony, D. (2014). The energetics of a Malagasy rodent, *Macrotarsomys ingens* (Nesomyinae): a test of island and zoogeographical effects on metabolism. *J. Comp. Physiol. B.* 184(8), 1077-1089. doi: 10.1007/s00360-014-0853-9.
- Lovegrove, B.G. (2000). The zoogeography of mammalian basal metabolic rate. *Am. Nat.* 156(2), 201-219. doi: 10.1086/303383.
- Lovegrove, B.G. (2012a). The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol. Rev.* 87(1), 128-162. doi: 10.1111/j.1469-185X.2011.00188.x.
- Lovegrove, B.G. (2012b). The evolution of mammalian body temperature: the Cenozoic supraendothermic pulses. *J. Comp. Physiol. B.* 182(4), 579-589. doi: 10.1007/s00360-011-0642-7.
- Lovegrove, B.G. (2012c). "A single origin of heterothermy in mammals," in *Living in a seasonal world: Thermoregulatory and metabolic adaptations*, eds. T. Ruf, C. Bieber, W. Arnold & E. Millesi. (Berlin, Heidelberg: Springer Berlin Heidelberg), 3-11.
- Lovegrove, B.G. (2017). A phenology of the evolution of endothermy in birds and mammals. *Biol. Rev.* 92. doi: 10.1111/brv.12280.
- Lovegrove, B.G., Canale, C., Levesque, D.L., Fluch, G., Rehakova-Petru, M., and Ruf, T. (2014a). Are tropical small mammals physiologically vulnerable to arrhenius effects and climate change? *Physiol. Biochem. Zool.* 87(1), 30-45. doi: 10.1086/673313.
- Lovegrove, B.G., and Génin, F. (2008). Torpor and hibernation in a basal placental mammal, the Lesser Hedgehog Tenrec *Echinops telfairi*. *J. Comp. Physiol. B.* 178(6), 691-698. doi: 10.1007/s00360-008-0257-9.
- Lovegrove, B.G., Lobban, K.D., and Levesque, D.L. (2014b). Mammal survival at the Cretaceous–Palaeogene boundary: metabolic homeostasis in prolonged tropical hibernation in tenrecs. *Proc. R. Soc. B.* 281(1796), 20141304. doi: 10.1098/rspb.2014.1304.

- Lovegrove, B.G., and Mowoe, M.O. (2014). The evolution of micro-cursoriality in mammals. *J. Exp. Biol.* 217(Pt 8), 1316-1325. doi: 10.1242/jeb.095737.
- Luken, J.O., Kuddes, L.M., and Tholemeier, T.C. (1997). Response of understory species to gap formation and soil disturbance in *Lonicera maakii* thickets. *Restor. Ecol.* 5(3), 229-235. doi: 10.1046/j.1526-100X.1997.09727.x.
- MacKinnon, J., and MacKinnon, K. (1980). The behavior of wild spectral tarsiers. *Int. J. Primatol.* 1(4), 361-379. doi: 10.1007/bf02692280.
- Malhi, Y. (2012). The productivity, metabolism and carbon cycle of tropical forest vegetation. *J. Ecol.* 100(1), 65-75. doi: 10.1111/j.1365-2745.2011.01916.x.
- Martínez-Ramos, M., Ortiz-Rodríguez, I.A., Piñero, D., Dirzo, R., and Sarukhán, J. (2016). Anthropogenic disturbances jeopardize biodiversity conservation within tropical rainforest reserves. *Proc. Natl. Acad. Sci. U. S. A.* 113(19), 5323-5328. doi: 10.1073/pnas.1602893113.
- McCarty, J.P. (2001). Ecological consequences of recent climate change. *Conserv. Biol.* 15(2), 320-331. doi: 10.1046/j.1523-1739.2001.015002320.x.
- McKechnie, A.E., and Wolf, B.O. (2004). Partitioning of evaporative water loss in white-winged doves: plasticity in response to short-term thermal acclimation. *J. Exp. Biol.* 207(2), 203-210. doi: 10.1242/jeb.00757.
- McNab, B.K. (1978). The evolution of endothermy in the phylogeny of mammals. *Am. Nat.* 112(983), 1-21. doi: <http://www.jstor.org/stable/2460134>.
- McNab, B.K. (1985). "Energetics, population biology, and distribution of Xenarthrans, living and extinct.," in *The evolution and ecology of armadillos, sloths and Vermilinguas*, ed. G.G. Montgomery. (Washington, DC: Smithsonian Institution Press), 219-232.
- McNab, B.K. (1989). Temperature regulation and rate of metabolism in 3 Bornean bats. *J. Mammal.* 70(1), 153-161. doi: 10.2307/1381678.
- McNab, B.K., and Wright, P.C. (1987). Temperature regulation and oxygen consumption in the Philippine tarsier *Tarsius syrichta*. *Physiol. Zool.* 60(5), 596-600. doi: 10.2307/30156133.
- Meredith, R.W., Janecka, J.E., Gatesy, J., Ryder, O.A., Fisher, C.A., Teeling, E.C., et al. (2011). Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification. *Science* 334(6055), 521-524. doi: 10.1126/science.1211028.

- Miettinen, J., Shi, C., and Liew, S.C. (2016). Land cover distribution in the peatlands of Peninsular Malaysia, Sumatra and Borneo in 2015 with changes since 1990. *Glob. Ecol. Conserv.* 6, 67-78. doi: <http://dx.doi.org/10.1016/j.gecco.2016.02.004>.
- Mora, C., Dousset, B., Caldwell, I.R., Powell, F.E., Geronimo, R.C., Bielecki, C.R., et al. (2017). Global risk of deadly heat. *Nat. Clim. Change* 7(7), 501-506. doi: 10.1038/nclimate3322.
- Morley, R.J. (2000). *Origin and evolution of tropical rain forests*. New York: John Wiley & Sons.
- Neri-Arboleda, I., Stott, P., and Arboleda, N.P. (2002). Home ranges, spatial movements and habitat associations of the Philippine tarsier (*Tarsius syrichta*) in Corella, Bohol. *J. Zool.* 257(3), 387-402. doi: 10.1017/S0952836902000997.
- Nowack, J., Stawski, C., and Geiser, F. (2017). More functions of torpor and their roles in a changing world. *J. Comp. Physiol. B.* 187(5), 889-897. doi: 10.1007/s00360-017-1100-y.
- Numerikal, J.M.M.S.R.C. (2009). *Climate Change Scenarios for Malaysia, 2001-2099*. Jabatan Meteorologi Malaysia.
- O'Leary, M.A., Bloch, J.I., Flynn, J.J., Gaudin, T.J., Giallombardo, A., Giannini, N.P., et al. (2013). The placental mammal ancestor and the post-K-Pg radiation of placentals. *Science* 339(6120), 662-667. doi: 10.1126/science.1229237.
- Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Systemat.* 37, 637-669. doi: 10.2307/30033846.
- Pearson, R.G., and Dawson, T.P. (2003). Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecol. Biogeogr.* 12, 361-371.
- Prothero, J.W. (2015). *The design of mammals: A scaling approach*. Cambridge, UK: Cambridge University Press.
- Pyle, J.A., Warwick, N.J., Harris, N.R.P., Abas, M.R., Archibald, A.T., Ashfold, M.J., et al. (2011). The impact of local surface changes in Borneo on atmospheric composition at wider spatial scales: coastal processes, land-use change and air quality. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366(1582), 3210-3224. doi: 10.1098/rstb.2011.0060.
- Racey, P.A., and Stephenson, P.J. (1996). "Reproductive and energetic differentiation of the Tenrecidae of Madagascar," in *Biogéographie de Madagascar*, ed. L.W. R. (Orstom, Paris), 307-319.

- Reynolds, G., Payne, J., Sinun, W., Mosigil, G., and Walsh, R.P.D. (2011). Changes in forest land use and management in Sabah, Malaysian Borneo, 1990–2010, with a focus on the Danum Valley region. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366(1582), 3168-3176. doi: 10.1098/rstb.2011.0154.
- Ricklefs, R.E., and Wikelski, M. (2002). The physiology/life-history nexus. *Trends Ecol. Evol.* 17(10), 462-468. doi: 10.1016/s0169-5347(02)02578-8.
- Roberts, T.E., Lanier, H.C., Sargis, E.J., and Olson, L.E. (2011). Molecular phylogeny of treeshrews (Mammalia: Scandentia) and the timescale of diversification in Southeast Asia. *Mol. Phylogenet. Evol.* 60(3), 358-372. doi: <https://doi.org/10.1016/j.ympev.2011.04.021>.
- Rohde, K. (1992). Latitudinal Gradients in Species Diversity: The Search for the Primary Cause. *Oikos* 65(3), 514-527. doi: 10.2307/3545569.
- Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C., and Pounds, J.A. (2003). Fingerprints of global warming on wild animals and plants. *Nature* 421(6918), 57-60. doi: 10.1038/nature01333.
- Rosenzweig, C., Karoly, D., Vicarelli, M., Neofotis, P., Wu, Q., Casassa, G., et al. (2008). Attributing physical and biological impacts to anthropogenic climate change. *Nature* 453(7193), 353-357. doi:http://www.nature.com/nature/journal/v453/n7193/supinfo/nature06937_S1.html.
- Rowe, T.B., Macrini, T.E., and Luo, Z.-X. (2011). Fossil evidence on origin of the mammalian brain. *Science* 332(6032), 955-957. doi: 10.1126/science.1203117.
- Ruddell, D.M., Harlan, S.L., Grossman-Clarke, S., and Buyantuyev, A. (2010). "Risk and exposure to extreme heat in microclimates of Phoenix, AZ," in *Geospatial Techniques in Urban Hazard and Disaster Analysis*, eds. P.S. Showalter & Y. Lu. Springer Netherlands), 179-202.
- Schmidt-Nielsen, K. (1997). *Animal physiology: adaptation and environment*. Cambridge: Cambridge University Press.
- Schmitz, J., Ohme, M., and Zischler, H. (2001). SINE insertions in cladistic analyses and the phylogenetic affiliations of *Tarsius bancanus* to other primates. *Genetics* 157(2), 777-784.
- Scotese, C.R. (1991). Jurassic and cretaceous plate tectonic reconstructions. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 87(1), 493-501. doi: [https://doi.org/10.1016/0031-0182\(91\)90145-H](https://doi.org/10.1016/0031-0182(91)90145-H).

- Sherwood, S.C., and Huber, M. (2010). An adaptability limit to climate change due to heat stress. *Proc. Natl. Acad. Sci. U. S. A.* 107(21), 9552-9555. doi: 10.1073/pnas.0913352107.
- Simons, E.L. (2003). "The fossil record of Tarsier evolution," in *Tarsiers: past, present, and future*, eds. P.C. Wright, E.L. Simons & S. Gursky. (New Brunswick: Rutgers University Press), 9-34.
- Stawski, C., and Geiser, F. (2012). Will temperature effects or phenotypic plasticity determine the thermal response of a heterothermic tropical bat to climate change? *PLOS ONE* 7(7), e40278. doi: 10.1371/journal.pone.0040278.
- Struebig, M.J., Wilting, A., Gaveau, D.L., Meijaard, E., Smith, R.J., Fischer, M., et al. (2015). Targeted conservation to safeguard a biodiversity hotspot from climate and land-cover change. *Curr. Biol.* 25(3), 372-378. doi: 10.1016/j.cub.2014.11.067.
- Tan, K.H., Zubaid, A., and Kunz, T.H. (1998). Food habits of *Cynopterus brachyotis* (Muller) (Chiroptera: Pteropodidae) in Peninsular Malaysia. *J. Trop. Ecol.* 14(3), 299-307.
- Tattersall, G.J., Sinclair, B.J., Withers, P.C., Fields, P.A., Seebacher, F., Cooper, C.E., et al. (2012). Coping with thermal challenges: Physiological adaptations to environmental temperatures. *Compr. Physiol.* 2, 2151-2202. doi: 10.1002/cphy.c110055.
- Toledo, N., Bargo, M.S., Vizcaíno, S.F., De Iuliis, G., and Pujos, F. (2017). Evolution of body size in anteaters and sloths (Xenarthra, Pilosa): phylogeny, metabolism, diet and substrate preferences. *Earth Environ Sci Trans R Soc Edinb* 106(4), 289-301. doi: 10.1017/S1755691016000177.
- Tuff, K.T., Tuff, T., and Davies, K.F. (2016). A framework for integrating thermal biology into fragmentation research. *Ecol. Lett.* 19, 361-374. doi: 10.1111/ele.12579.
- Turbill, C., Bieber, C., and Ruf, T. (2011). Hibernation is associated with increased survival and the evolution of slow life histories among mammals. *Proc. R. Soc. B.* 278(1723), 3355-3363. doi: 10.1098/rspb.2011.0190.
- Turner, I., and Corlett, R.T. (1996). The conservation value of small, isolated fragments of lowland tropical rain forest. *Trends Ecol. Evol.* 11(8), 330-333.
- Walsberg, G.E. (2000). Small Mammals in hot deserts: Some generalizations revisited. *BioScience* 50(2), 109-120. doi: 10.1641/0006-3568(2000)050[0109:SMIHDS]2.3.CO;2.

- Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., et al. (2002). Ecological responses to recent climate change. *Nature* 416(6879), 389-395. doi: 10.1038/416389a.
- Warwick, N.J., Archibald, A.T., Ashworth, K., Dorsey, J., Edwards, P.M., Heard, D.E., et al. (2013). A global model study of the impact of land-use change in Borneo on atmospheric composition. *Atmos. Chem. Phys.* 13(18), 9183-9194. doi: 10.5194/acp-13-9183-2013.
- Wegener, A. (1912). Die Entstehung der Kontinente. *Geologische Rundschau* 3(4), 276-292. doi: 10.1007/bf02202896.
- Welbergen, J.A., Klose, S.M., Markus, N., and Eby, P. (2008). Climate change and the effects of temperature extremes on Australian flying-foxes. *Proc. R. Soc. B.* 275(1633), 419-425. doi: 10.1098/rspb.2007.1385.
- Werth, D., and Avissar, R. (2002). The local and global effects of Amazon deforestation. *J. Geophys. Res.* 107(D20), LBA 55-51-LBA 55-58. doi: 10.1029/2001JD000717.
- Wiens, J.A., Stralberg, D., Jongsomjit, D., Howell, C.A., and Snyder, M.A. (2009). Niches, models, and climate change: assessing the assumptions and uncertainties. *Proc. Natl. Acad. Sci. U. S. A.* 106(Suppl 2), 19729-19736. doi: 10.1073/pnas.0901639106.
- Wiens, J.J., and Graham, C.H. (2005). Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu. Rev. Ecol. Evol. Systemat.* 36(1), 519-539. doi: 10.1146/annurev.ecolsys.36.102803.095431.
- Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A., and Langham, G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *PLOS Biol.* 6(12), e325. doi: 10.1371/journal.pbio.0060325.
- Withers, P.C., Cooper, C.E., and Nespolo, R.F. (2012). Evaporative water loss, relative water economy and evaporative partitioning of a heterothermic marsupial, the monito del monte (*Dromiciops gliroides*). *J. Exp. Biol.* 215(16), 2806-2813. doi: 10.1242/jeb.070433.

Chapter Two - Searching for the Haplorrhine Heterotherm: Free-ranging Tarsier Data

Shaun Welman^{1*}, Andrew A. Tuen² and Barry G. Lovegrove¹

¹ School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

² Institute of Biodiversity and Environmental Conservation, Universiti Malaysia, Sarawak, Malaysia

*Corresponding author: welmans@ukzn.ac.za

Note: The manuscript style has been formatted in British English

Abstract

The observation of adaptive heterothermy in a single suborder (Strepsirrhini) only within the primates is puzzling. Given that the placental-mammal ancestor was likely a heterotherm, we explored the potential for adaptive heterothermy (torpor or hibernation) in a primate closely related to the Strepsirrhini. Based upon phylogeny, body size and habitat stability since the Late Eocene, we selected western tarsiers (*Cephalopachus bancanus*) from the island of Borneo. Being the sister clade to Strepsirrhini and basal in Haplorrhini (monkeys and apes), we hypothesized that *C. bancanus* might have retained the heterothermic capacity observed in several small strepsirrhines. We measured resting metabolic rate, subcutaneous temperature, evaporative water loss and the percentage of heat dissipated through evaporation, at ambient temperatures (T_a) between 22 - 35°C in fresh-caught wild animals (126.1 ± 2.4 g). We also measured core body temperatures in free-ranging animals. The thermoneutral zone (TNZ) was 25 - 30°C and the basal metabolic rate was 3.52 ± 0.06 W.kg⁻¹ (0.65 ± 0.01 ml O₂.g⁻¹.hr⁻¹). There was no evidence of adaptive heterothermy in either the laboratory data or the free-ranging data. Instead, animals appeared to be cold sensitive because they could not defend normothermia ($T_b \sim 35$ °C) below TNZ, despite efforts to increase metabolic heat production. We discuss possible reasons for the apparent lack of adaptive heterothermy in tarsiers, and identify putative heterotherms within Platyrrhini. We also document our concern for the vulnerability of *C. bancanus* to future temperature increases associated with global warming.

Keywords: Metabolism, primate thermoregulation, tropics, evolution, tarsiers

Introduction

The capacity to employ adaptive heterothermy, meaning that the animal can use torpor or hibernation, conveys significant fitness benefits and promotes survivability (Geiser and Turbill, 2009; Geiser and Brigham, 2012; Lovegrove et al., 2014b; Nowack et al., 2015; Stawski et al., 2015; Nowack et al., 2016; Lovegrove, 2017). These benefits may be derived either as a direct consequence of the reduction in energy expenditure and, in the case of hibernators specifically, the preservation of fat reserves during periods of low resource availability (Lovegrove, 2000; Dausmann, 2014), or, indirectly by reducing the risk of predation due to decreased foraging effort (Bieber and Ruf, 2009; Stawski and Geiser, 2010; Bieber et al., 2014). Adaptive heterothermy may also indirectly benefit reproduction by manipulating foetal growth rate or by enhancing sperm storage (reviewed in Geiser and Brigham, 2012). However, whereas the benefits of adaptive heterothermy may be well documented, its origin remains hotly debated (Crompton et al., 1978; McNab, 1978; Bennett and Ruben, 1979; Hayes and Garland, 1995; Farmer, 2000; Koteja, 2000; Grigg et al., 2004; Kemp, 2006; Clarke and Pörtner, 2010; Lovegrove, 2012b; a; 2017).

For many years, the role of adaptive heterothermy seemed to have been an afterthought in discussions about the evolution of endothermy (Bennett and Ruben, 1979; Bennett, 1991; Hayes and Garland, 1995). Traditionally, it would appear, strict homeothermy was regarded as having evolved directly from the ectothermic ancestral state, and adaptive heterothermy evolved thereafter. However, with the aid of maximum likelihood character state reconstruction, Lovegrove (2012a) determined that the last common mammalian ancestor was likely to have displayed adaptive heterothermy and that strict homeothermy was an apomorphic state. This conclusion supported arguments by Augee and Gooden (1992) and later by Grigg et al. (2004), who argued that the role of adaptive heterothermy in the evolution of endothermy was greatly underappreciated. Further, the likelihood that adaptive heterothermy is plesiomorphic, in combination with recent work on brown antechinus (*Antechinus stuartii*; Stawski et al., 2015), sugar gliders (*Petaurus breviceps*; Nowack et al., 2015), and short-beaked echidnas (*Tachyglossus aculeatus*; Nowack et al., 2016), provided support for the argument that the ancestors of the three crown mammalian clades namely, the Monotremata, Marsupialia and Placentalia, likely only survived the mass extinction event marking the Cretaceous-Paleogene (K-Pg) boundary because of their heterothermic capacity (Lovegrove et al., 2014b; Lovegrove, 2017).

For many decades, it appeared that adaptive heterothermy within the primates was geographically restricted to a single family - the Cheirogaleidae of Madagascar (see Dausmann, 2008). Now, in addition to observations of torpor and hibernation in Malagasy

mouse lemurs (Ortmann et al., 1997; Aujard et al., 1998; Schmid and Kappeler, 1998; Schmid, 2000; Kobbe and Dausmann, 2009; Schmid and Ganzhorn, 2009) and hibernation in dwarf lemurs (Dausmann et al., 2000; Dausmann et al., 2005), adaptive heterothermy has been observed in two non-Malagasy primates. Torpor, despite the initial lack of evidence for it (Knox and Wright, 1989; Mzilikazi et al., 2006), occurs in the African lesser bushbaby (*Galago maholi*; Nowack et al., 2010), albeit under extreme conditions only. Hibernation has now also been observed in the pygmy slow loris in Vietnam (*Nycticebus pygmaeus*; Ruf et al., 2015). Even with the additional observations of adaptive heterothermy in the Galagidae and Lorisidae, all observations within Primates remain within the Strepsirrhini clade prompting the question why it has not also been observed in the haplorrhines? Has adaptive heterothermy potentially been ‘lost’ in more derived primate clades or does its absence reflect an artefact of sampling bias?

In this paper, we sought to explore the potential for adaptive heterothermy in a non-strepsirrhine primate in an attempt to gain further insight into the primate adaptive heterothermy phenotype. Our choice of study animal was determined by three principal factors; a) close phylogenetic relatedness to the strepsirrhines, b) an insular tropical existence, and c) small body size. Choosing a close relative provides the best opportunity to confirm a potential retention of the ancestral adaptive heterothermy condition. The island existence and small body size criteria stem from observations of extensive employment of adaptive heterothermy by small-bodied Malagasy lemurs and because most heterothermic mammals are small (< 1kg) (Geiser, 1998; Lovegrove, 2012a; Ruf and Geiser, 2015). It has been proposed that, in general, mammals that colonised tropical islands during the Early Cenozoic, that is, prior to the onset of global cooling during the Late Eocene, retained plesiomorphic climate-adaptation traits through stabilising selection (Hansen, 1997; Lovegrove, 2012a; Lobban et al., 2014).

Based on the aforementioned criteria, the most *apropos* model to search for the evidence of adaptive heterothermy in non-strepsirrhine primates is the tarsier (Tarsiidae). Tarsiers are small (80 – 150g; but see Clarke, 1943), tropical, nocturnal and arboreal primates which inhabit the forests of South-east Asia (MacKinnon and MacKinnon, 1980; Crompton and Andau, 1986; 1987; Neri-Arboleda et al., 2002; Groves and Shekelle, 2010). The reported body temperatures (T_b) of 33.3°C (Lovegrove et al., 2014a) and 33.8°C (McNab and Wright, 1987) for the Philippine tarsier (*Tarsius syrichta*) show that they are “basoendotherms” i.e. T_b < 35°C and thus similar to the predicted ancestral condition (*sensu* Lovegrove, 2012a); *ca.* 3°C lower than the average T_b of the primate clade (see Clarke et al., 2010). Since their evolution during the Eocene, tarsiers have persisted within a continuously tropical environment in habitats that are argued to be very similar to those that their ancestors

inhabited; despite some changes in floristic composition (Jablonski, 2003; Simons, 2003). They are also the only strictly insectivorous/carnivorous primate and, as a rule, take live prey (Jablonski and Crompton, 1994; Gursky, 2000). With regards to their phylogeny, their position has been hotly debated and shuffled around the primate tree (Schwartz, 1984; Schmitz et al., 2001; Meireles et al., 2003; Simons, 2003; Yoder, 2003; Matsui et al., 2009; Perelman et al., 2011). However, the most recent study by Hartig et al. (2013) supports the Haplorrhini hypothesis i.e. that tarsiers are the sister taxa to the Anthropoids, as originally proposed by Pocock (1918). Tarsiers are thus the closest extant relatives to the strepsirrhines.

Given the close phylogenetic relationship of tarsiers to the Strepsirrhini, as well as the varied observations of torpor in the Strepsirrhini (Dausmann, 2014; Dausmann and Warnecke, 2016), we predicted that stabilizing selection may have favoured the retention of the plesiomorphic capacity for adaptive heterothermy within tarsiers (Lovegrove, 2012a). Currently, there is no physiological evidence in tarsiers that supports our prediction. Most of our understanding of tarsier thermoregulation does however stem from three studies on *T. syrichta* (Clarke, 1943; McNab and Wright, 1987; Lovegrove et al., 2014a), but it has been speculated that western tarsiers (*Cephalopachus bancanus*, previously *T. bancanus*) may be capable of torpor (Niemitz, 1984; Niemitz et al., 1984).

Our study had two main objectives. The first was to determine the thermoregulatory response of wild-caught *C. bancanus* to varying ambient temperatures (T_a), noting any potential indication of hypometabolism or the associated reduction in T_b . The second was to document free-ranging core temperatures (T_{core}) continuously over several months to determine whether torpor occurs in their natural setting.

Materials and methods

Animal capture and husbandry

Nine male and four non-pregnant female adult tarsiers were used in the study. Animals were captured in mist nets during two sampling periods between August - October 2014 and March - August 2015, at Sama Jaya Nature Reserve (1°31'16" N; 110°23'15" E) on the island of Borneo. Unlike temperate zones or other higher latitudes, Southeast Asia does not have typical summer or winter seasons. Instead, the region remains warm throughout the year, but rainfall patterns are heavily influenced by the phases of the El Niño Southern Oscillations. Although periods of El Niño cause an overall reduction in the mean annual rainfall of the broader state of Sarawak, at a local scale, the mean annual rainfall of the area around the study site seemingly remains high throughout (ca. 3700 mm per annum; Sa'adi et al., 2017).

The vegetation type of the study area was secondary forest and the reserve encompassed an area of approximately 38 hectares. The number of daylight hours remained fairly constant, with sunrise typically occurring shortly after 06:00 and sunset occurring shortly before 19:00. Nets were set in areas with a dense concentration of narrow-stemmed trees, as well as in areas with notable olfactory cues from scent markings of resident tarsiers. All nets were opened at dusk (ca. 18:00) and checked at regular intervals throughout the night during the tarsier's active phase (α -phase). Captured individuals were sexed and thereafter weighed using an electronic scale (Shimadzu TX3202L, Columbia, USA). Temperature-sensitive passive integrated transponder (PIT) tags (Biomark HDX12, Boise, Idaho, USA) were injected into their flanks. The PIT tags enabled us to measure the animals' subcutaneous body temperatures (T_{sub}) and also provided a unique identification code. The PIT tag's location was chosen to avoid any potential harm to their vital organs, while still being situated in close proximity to their core region during normal posture. After the injection, the animals were rehydrated and housed in a covered wire mesh cage fitted with branches. During captivity, animals were fed live lizards or crickets and provided with water *ad libitum*. However, to ensure a post-absorptive status during measurements, all animals received their last meal six hours prior to respirometry measurements. Tarsiers are notoriously difficult to maintain in captivity so all animals were held captive for a maximum of 36 hours only.

All experimental procedures were reported to and approved by the University of Kwazulu-Natal Animal Ethics Committee (116/13/Animal), which adopts the guidelines described by the Canadian Council on Animal Care. All experimental protocols were also approved by the Sarawak Forestry Department [permit number: NCCD.907.4.4(9)-223, NCCD.907.4.4(13)-277].

Gas exchange measurements

We used the incurrent flow measurement flow-through respirometry design described by Lighton (2008) to measure the rate of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and evaporative water loss (EWL) of tarsiers exposed to varying T_{a} s. Animals were housed in sealed 4l respirometers that were constructed from clear plexiglass acrylic. Respirometers were fitted with a grid platform elevated above a 1 cm deep layer of mineral oil used to trap urine and faeces. Dried and CO_2 -free air was flowed through the chamber at constant rates between 400 – 500 $\text{ml}\cdot\text{min}^{-1}$, sufficient to maintain O_2 concentrations of 20.8 – 20.0% within the respirometer. Incurrent air was dried and scrubbed of CO_2 by drawing it through a PC-4 Condensing Dryer (Sable Systems, Las Vegas, USA), followed by a column of silica gel and a column of indicating soda lime, and finally a column of indicating Drierite before reaching the pump and flow meter unit (SS-4 sub-sampler, Sable

Systems). With the aid of a RM-8 Flow Multiplexer (Sable Systems), the excurrent air from the animal chamber and a stream of reference air were sequentially subsampled and passed through a series of gas analysers. The water vapour content of the air was measured using a RH-300 water vapour analyser (Sable Systems), whereafter it was dried again using a column of Drierite and flowed through a field gas analysis system (Foxbox-C, Sable Systems) to measure the fractional concentrations of CO₂ and O₂. CO₂ was scrubbed from the air stream using soda lime prior to the O₂ analyser. Sable System's data acquisition software, Expedata (v 1.7.22), was used to interpret and record the digital outputs from the equipment using a laptop at 1-sec intervals. The respective rates of EWL, $\dot{V}\text{CO}_2$ and $\dot{V}\text{O}_2$ were calculated using the equations presented in Withers (2001).

The O₂ analyser was spanned prior to every measurement and the water vapour and CO₂ gas analysers were calibrated monthly. Compressed pure nitrogen gas was used to set the zero point during all calibrations. A bubbler flask and waterbath were used to generate humid air of a known dew point to set the water vapour span values. The CO₂ span value was set using certified commercially available compressed CO₂ gas.

Experimental protocol for gaseous exchange measurements

We determined the animals' thermoregulatory response by concurrently measuring resting metabolic rate (RMR) and T_{sub} adjustments while exposed to temperatures between 22 -35°C. The air temperature within the respirometers was measured using commercially available temperature sensitive data-loggers (iButton DS1922L, Thermochron, Dallas, TX, USA; resolution: 0.0625°C), hereafter temperature loggers. The experimental temperatures were controlled by partially submerging the respirometers into a body of water within a modified coolerbox, wherein the temperature of the water was regulated using a waterbath via a closed loop, such that the water flowed from the waterbath into the coolerbox and back into the waterbath. A Biomark HPR plus reader and antennae were used to read and record T_{sub} every 30 sec; providing a real-time visualization of the animals' thermal profile during measurements. Temperature loggers and PIT tags were calibrated following the method of Toussaint and McKechnie (2012).

All measurements were performed on solitary individuals during their rest phase (p-phase; 06:00 - 18:00). Tarsiers were exposed to a single temperature for 3 - 6 hours, and each tarsier was exposed to a maximum of two temperatures per capture. In cases where tarsiers were exposed to two temperatures, the experimental temperatures and order of exposure were allocated randomly. To minimize the bias of any particular tarsier, all tarsiers were only exposed to a maximum of 3 temperatures, irrespective of the frequency of recapture. Also, there were two instances where measurements were terminated prematurely because the

individuals remained restless and their T_{sub} s increased rapidly to 38°C; both occurred at $T_a = 35^\circ\text{C}$. Data from these two individuals were excluded from analyses.

Surgical procedure and free-ranging body temperature measurements

After the completion of respirometry measurements, five of the 13 animals (three males and two females) used in the respirometry study, were surgically implanted with the same custom designed temperature sensitive data-loggers (MCP 9800, Microchip Technology, Chandler, AZ; resolution: 0.0625°C) described in Lovegrove et al. (2014a) to measure T_{core} in free-ranging animals. The loggers were assembled on site at our research station at the Universiti Malaysia Sarawak. A 3-volt CR 1632 coin battery was soldered to the terminals of the logger circuit board and the units were then coated with an acrylic protective lacquer (Electrolube, HK Wentworth Ltd., Leicestershire, UK) and encapsulated in surgical wax; yielding a final weight of ca. 4g. In compliance with the conditions stipulated for the approval of permits, all surgical procedures were performed by a local veterinarian (Dr. Samuel Kiyui, Malaysian Veterinary Council registration number: 049) under sterile conditions at his surgery. Anaesthesia was induced by a 1ml intramuscular injection of a cocktail containing Tiletamine and Zolazepam as active ingredients (Zoletil 100, Virbac Veterinary Pharmaceuticals), whereafter the data loggers were inserted into the peritoneal cavity via an incision along the *linea alba*. The incision was sutured using 3/0 absorbable Dexon polyglycolic acid suture and a topical antiseptic [Chlorhexidine gluconate 5% (w/v) and Isopropyl alcohol 3.15% (w/v)] was liberally applied to the area. While anaesthetized, animals were also fitted with external VHF radio-telemetry transmitter collars (PD-2C, Holohil Systems Ltd., Ottawa, Canada; weight = 4g) to allow us to track and monitor the animals. Tarsiers were released at their site of capture as soon as possible once they had regained their mental acuity and monitored for the first hour following release. Thereafter, daily observations were made for the first week and then monitoring became periodical to minimize disturbance.

The T_{core} data loggers were calibrated in a similar manner to that previously mentioned. They were programmed to record readings every 30 min for a period of six months. Of the five implants, we recovered three and five weeks of data from a male and female respectively. The unsuccessful attempts were due to premature battery failure caused by the soldering process.

The T_a within the forest was recorded throughout the study period using the same type of temperature loggers that were used during metabolic measurements. The loggers were housed in black and white solar radiation shields that were attached as a pair (1 black and 1 white) at a height of 1.5m above the ground to three trees located in different sections of the reserve. All three trees were observed tarsier rest sites. All plots and analyses of T_a at the field site were made using the mean T_a from all six loggers.

Data analyses

Laboratory measurements

Baseline and lag corrections were performed in Expedata to synchronize the respective gas-exchange traces before any calculations were made. RMR and EWL were calculated from steady-state traces corresponding to the most level continuous 10-min section of the $\dot{V}O_2$ trace identified using Expedata functions. The accompanying body mass (M_b) was calculated using a regression of the animals' M_b at the start and end of metabolic measurements. Metabolic rate (in Watts) was calculated by converting the respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$) using the thermal equivalence data in Table 4.2 in Withers (1992). Evaporative heat loss (in Watts) was calculated assuming $2.26 \text{ J. mg H}_2\text{O}^{-1}$, and then used to calculate the amount of metabolic heat dissipated through evaporation as the ratio of evaporative heat loss to metabolic heat production (EHL/MHP).

To objectively identify inflection points along the profiles of RMR, T_{sub} , EWL and EHL/MHP as a function of T_a , we performed a piecewise regression analysis in R 3.0.2 (R Core Team, 2017) using the package 'segmented' (Muggeo, 2008). Each section of data identified through the analysis was treated as independent and we adopted the approach of implementing mixed-effect models using the R package 'nlme' (Pinheiro et al., 2016) to evaluate the effect of T_a while accounting for other variables. We developed models using various permutations of M_b and sex as fixed factors. To account for repeated measurements in laboratory measurements ($n = 13$) we included individual ID as a random factor in all of the models. We then compared their Akaike Information Criterion values that were corrected for small sample size (AICc and AICcWt; Burnham and Anderson, 2003) to determine the model of best fit using the R package "AICcmodavg" (Mazerolle, 2015). We tested for a sex difference in M_b at the time of first capture using a Student's t-test. All values are reported as mean \pm standard error unless stated otherwise. The assumptions of the models were verified in a similar manner to Levesque et al. (2014).

Free-ranging measurements.

We used Rayleigh's tests to assess whether there was any uniformity in the times at which maximum and minimum T_{core} in free-ranging animals ($n = 2$) and forest temperatures were observed. We also tested whether T_{core} followed a normally distributed pattern, using a Shapiro-Wilk's test.

Results

Thermoregulatory measurements

All thermoregulatory measurements were obtained concurrently during laboratory experiments using 13 wild-caught animals. The mean M_b at first capture was 126.1 ± 2.4 g. Males ($n = 9$, $M_b = 126.9 \pm 2.7$ g) were significantly heavier than females ($n = 4$, $M_b = 117.1 \pm 2.7$ g; one-tailed $t_{11} = 2.76$, $p = 0.009$).

Mass-specific RMR displayed inflection points at $T_a = 25.0 \pm 1.3^\circ\text{C}$ ($\pm 95\%$ confidence interval; CI) and $T_a = 30.0 \pm 2.5^\circ\text{C}$ ($\pm 95\%$ CI), and displayed a significant and linear relationship with $T_{as} < 25^\circ\text{C}$ ($F_{1,7} = 9.44$, $p < 0.018$, $r^2 = 0.57$) and $T_{as} > 30^\circ\text{C}$ ($F_{1,13} = 9.99$, $p = 0.007$, $r^2 = 0.43$), but not with T_{as} between these values (Fig. 2-1A, Table 2-1). Thus, the species' thermoneutral zone (TNZ) was *ca.* $25 - 30^\circ\text{C}$. We calculated a basal metabolic rate (BMR) of $3.52 \pm 0.06 \text{ W}\cdot\text{kg}^{-1}$ ($0.65 \pm 0.01 \text{ ml O}_2\cdot\text{g}^{-1}\cdot\text{hr}^{-1}$) by averaging all RMR values between and excluding those at the inflection points; including the values from both inflection points made no difference. The accuracy of the thermal limits proposed by the piecewise regression analysis was confirmed by substituting BMR into the respective regression equation of each line, to calculate the T_a at which they intersected BMR. The intersection corresponding to the lower limit of thermoneutrality (T_{lc}) was at $T_a = 25.2^\circ\text{C}$ and the upper limit (T_{uc}) was at $T_a = 30.6^\circ\text{C}$. The models which best predicted whole-animal RMR above (Akaike weight = 0.74) and below (Akaike weight = 0.70) TNZ included T_a as the only fixed factor (Table 2-2). In both cases, the next best models contained M_b as an additional factor, and explained all of the remaining Akaike weight for metabolism below TNZ. The remaining Akaike weight for metabolism above TNZ included gender as a factor.

T_{sub} displayed a single inflection point during the thermoregulatory measurements coincident with T_{uc} (Fig. 2-1B). Both sections of the T_{sub} profile had a significant and linear relationship with T_a ($T_a < 30^\circ\text{C}$: $F_{1,19} = 19.58$, $p < 0.001$; $T_a > 30^\circ\text{C}$: $F_{1,13} = 15.68$, $p = 0.002$), but the slope of the relationship for $T_a < 30^\circ\text{C}$ was lower (Table 2-1). The model which best predicted T_{sub} above T_{uc} included T_a as the only fixed factor (Akaike weight = 0.82), whereas the model best suited to predict T_{sub} below T_{uc} included all of the variables (Akaike weight = 0.73). The mean minimum T_{sub} was $32.4 \pm 0.5^\circ\text{C}$ observed at $T_a = 24^\circ\text{C}$, whereas at higher T_{as} there was no difference in the mean maximum T_{sub} observed at $T_a = 32^\circ\text{C}$ ($T_{sub} = 35.9 \pm 0.5^\circ\text{C}$) and $T_a = 33^\circ\text{C}$ ($T_{sub} = 36.0 \pm 0.5^\circ\text{C}$).

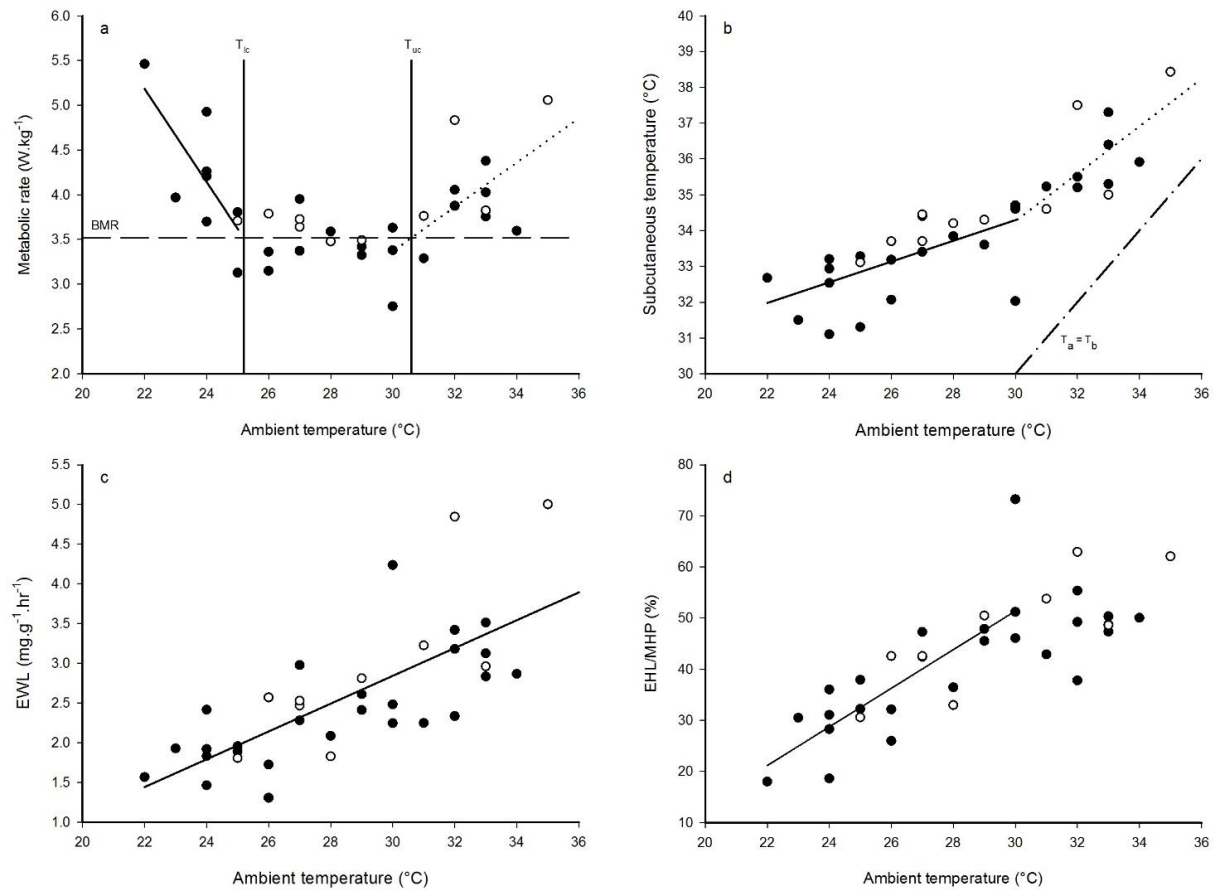


Figure 2-1: The thermoregulatory profile of *Cephalopachus bancanus*. a) Effect of ambient temperature on resting metabolic rate. b) Effect of ambient temperature on subcutaneous temperature. c) Effect of ambient temperature on the rate of evaporative water loss. d) Effect of ambient temperature on the amount of metabolic heat dissipated through evaporative cooling. Plotted regression lines indicate the best fit for the data based on piecewise regression analysis and the equations are presented in Table 2-1. Closed circles = males, open circles = females.

Table 2-1: Linear regression models describing the relationship between ambient temperature (T_a) and various physiological parameters in *Cephalopachus bancanus*. Mass-specific resting metabolic rate = RMR, subcutaneous temperature = T_{sub} , the rate of evaporative water loss = EWL, the amount of metabolic heat dissipated through evaporative cooling = EHL/MHP, * = $p < 0.05$, ** = $p < 0.01$.

Parameter	Temperature range	Regression equation
RMR	$T_a < 25^\circ\text{C}$:	$\text{RMR} = 16.65 - 0.5212 \times T_a^*$
	$T_a > 30^\circ\text{C}$:	$\text{RMR} = 0.2479 \times T_a - 4.069^{**}$
T_{sub}	$T_a < 30^\circ\text{C}$:	$T_{sub} = 25.64 + 0.2879 \times T_a^*$
	$T_a > 30^\circ\text{C}$:	$T_{sub} = 14.64 + 0.6547 \times T_a^*$
EWL	All	$\text{EWL} = 0.17512 \times T_a - 2.41254^*$
EHL/MHP	$T_a < 30^\circ\text{C}$:	$\text{EHL/MHP} = 3.761 \times T_a - 61.52^*$

Table 2-2: Linear mixed-effect models which best describe the significant relationships between ambient temperature (T_a) and resting metabolic rate (RMR), subcutaneous temperature (T_{sub}), the rate of evaporative water loss (EWL) and the amount of metabolic heat dissipated through evaporative cooling (EHL/MHP) in *Cephalopachus bancanus*. Only models with Akaike weights ≥ 0.01 are presented.

Fixed factors	Random factor	k	AICc	Akaike weights
RMR ($T_a > 30^\circ\text{C}$)				
T_a	~1 ID	4	-37.92	0.74
$T_a + Mb$	~1 ID	5	-34.89	0.16
$T_a + \text{Sex}$	~1 ID	5	-33.34	0.07
$T_a + Mb + \text{Sex}$	~1 ID	6	-31.08	0.02
RMR ($T_a < 25^\circ\text{C}$)				
T_a	~1 ID	4	-0.69	0.7
$T_a + Mb$	~1 ID	5	1.01	0.3
T_{sub} ($T_a > 30^\circ\text{C}$)				
T_a	~1 ID	4	51.57	0.82
$T_a + \text{Sex}$	~1 ID	5	56.01	0.09
$T_a + Mb$	~1 ID	5	56.15	0.08
$T_a + Mb + \text{Sex}$	~1 ID	6	61.34	0.01
T_{sub} ($T_a < 30^\circ\text{C}$)				
$T_a + Mb + \text{Sex}$	~1 ID	6	43.15	0.73
$T_a + Mb$	~1 ID	5	45.81	0.19
T_a	~1 ID	4	48.06	0.06
$T_a + \text{Sex}$	~1 ID	5	50.88	0.02
EWL				
T_a	~1 ID	4	117.26	0.58
$T_a + \text{Sex}$	~1 ID	5	119.66	0.17
$T_a + Mb$	~1 ID	5	119.67	0.17
$T_a + Mb + \text{Sex}$	~1 ID	6	121.47	0.07
EHL/MHP ($T_a < 30^\circ\text{C}$)				
T_a	~1 ID	4	-52.66	0.65
$T_a + Mb$	~1 ID	5	-50.19	0.19
$T_a + \text{Sex}$	~1 ID	5	-49.62	0.14
$T_a + Mb + \text{Sex}$	~1 ID	6	-46.19	0.03

EWL had a significant, positive linear relationship with T_a ($F_{1,34} = 37.9$, $p < 0.001$, $r^2 = 0.53$, Fig. 2-1C, Table 2-1) and was best predicted by a model containing T_a as the only fixed factor (Akaike weight = 0.58, Table 2-2). EWL ranged from a mean minimum of $1.86 \pm 0.37 \text{ mg}^{-1} \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$ at $T_a = 26^\circ\text{C}$, to a mean maximum of $3.44 \pm 0.52 \text{ mg}^{-1} \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$ at $T_a = 32^\circ\text{C}$. Barring the elevated rate at $T_a = 27^\circ\text{C}$, EWL remained $< 2 \text{ mg}^{-1} \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$ and was stable at $T_a \geq 28^\circ\text{C}$. In addition, EWL displayed a strong correlation with T_{sub} ($r = 0.81$), and T_{sub} was correlated to the amount of EHL/MHP ($r = 0.67$).

The percentage of EHL/MHP scaled linearly with T_a below T_{uc} ($F_{1,19} = 29.7$, $p < 0.001$, Fig. 2-1D, Table 2-1). At $T_a > 30^\circ\text{C}$, EHL/MHP remained relatively constant and no longer displayed any relationship with T_a . The mean maximum percentage of EHL/MHP was $57 \pm 6\%$ and was achieved at $T_a = 30^\circ\text{C}$. The model which best predicted EHL/MHP below T_{uc} was again the one which included T_a as the only fixed factor (Akaike weight = 0.65).

Free-ranging body temperature profile

Both tarsiers were implanted on the 19th June 2015, but we excluded all values prior to the 24th of June to minimize any potentially misleading data recorded during the period of recuperation.

The $T_{\text{core}}-T_a$ gradient was lowest during the p-phase and was $\leq 3^\circ\text{C}$ on 75% and 70% of the observation days for the male and female respectively; of those, 42% and 61% were $\leq 2^\circ\text{C}$ (Fig. 2-2A-B). The frequency distribution (0.5°C incremental bin category) of T_{core} did not conform to a Gaussian distribution pattern (♂ : $W = 0.98$, $p < 0.001$; ♀ : $W = 0.96$, $p < 0.001$), but displayed a slightly left-skewed unimodal pattern. For both individuals, the modal T_{core} was $35.0 \pm 0.5^\circ\text{C}$ (Fig. 2-3A-B). Furthermore, even though T_{core} often approached 37°C in the male, it only exceeded 37°C on one occasion, for an hour during the morning of the 8th July 2015. Quantile 1 of the free-ranging T_{core} s were similar between individuals (Q1: $\text{♂} = 34.5^\circ\text{C}$ vs Q1: $\text{♀} = 34.3^\circ\text{C}$) but the female displayed a higher propensity for T_{core} to decrease below Q1 (♀ : 16% of all T_{core} s vs ♂ : 8% of all T_{core} s). The mean daily range in T_{core} was 2.4°C for both individuals and the times at which the maximum and minimum T_a s and T_{core} s (excluding the maximum T_{core} of the male) were observed were not uniformly distributed throughout a 24-h period (Rayleigh's test, $p < 0.001$; Table 2-3). Peak T_{core} s during the α -phase occurred consistently at 19:00 or 06:00 which coincided with the highest and lowest scotophase T_a s respectively. The lowest T_{core} s were consistently observed at or before 10:00, preceding the onset of the rapid increase in T_a to the daily maximum (range: $27.9 - 35.2^\circ\text{C}$, Table 2-3) at ca. 14:30.

The frequency distribution of $T_{a,s}$ during the scotophase were slightly right skewed and unimodal, with a peak in frequency at $T_a = 25^\circ\text{C}$ (Fig. 2-4). The frequency distribution of $T_{a,s}$ during the photophase were left skewed and had a flat-shaped distribution; the highest frequencies were observed between $T_a = 30.0 - 32.5^\circ\text{C}$ (Fig 2-3).

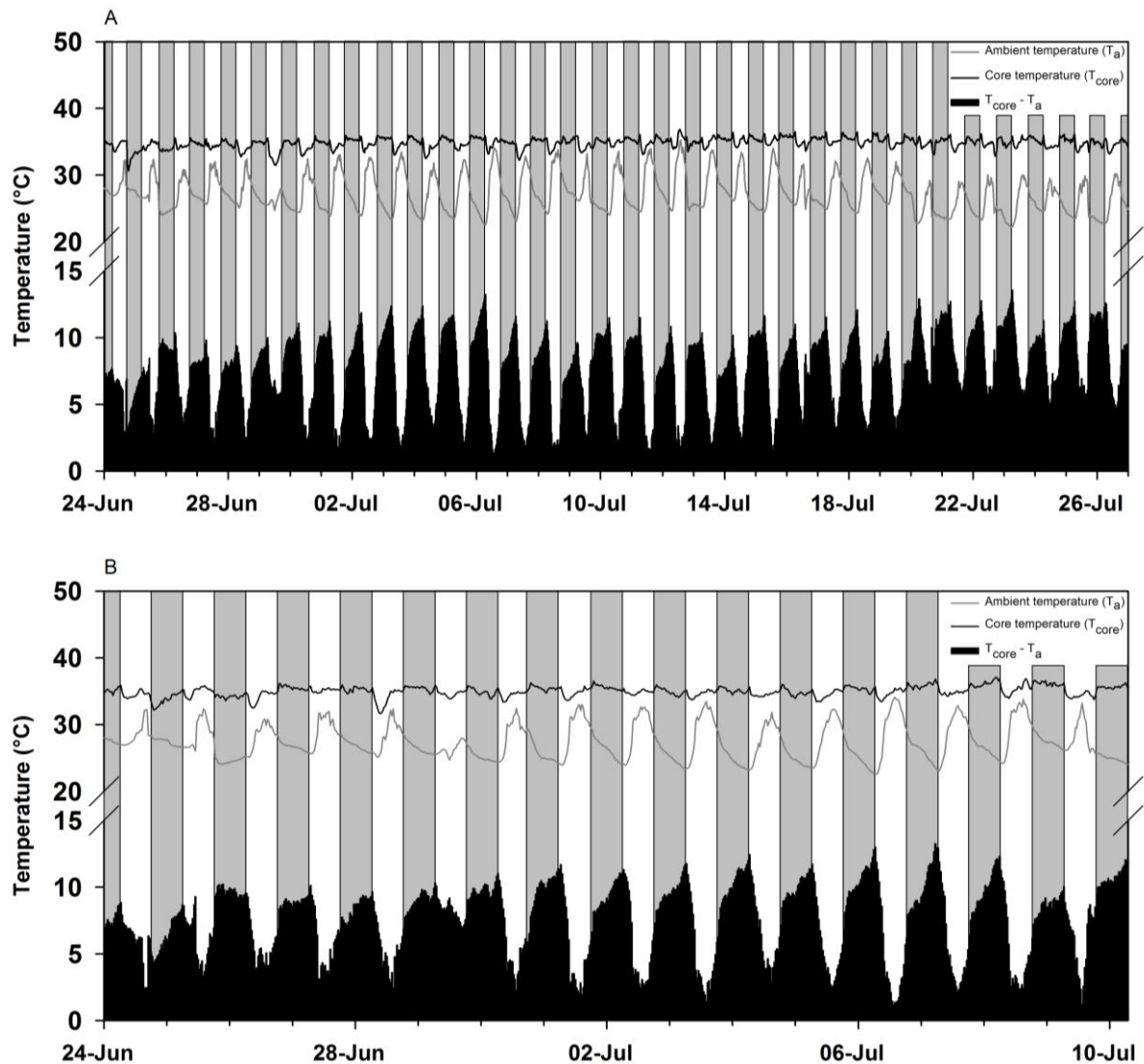


Figure 2-2: The temperature profile in free-ranging western tarsiers (*Cephalopachus bancanus*). A) The ambient temperatures (T_a ; grey line) and core temperatures (T_{core} ; black line) recorded in a female from 00:00 24th June 2015 to 00:00 27th July 2015. B) The ambient temperatures (T_a ; grey line) and core temperatures (T_{core} ; black line) recorded in a male from 00:00 24th June 2015 to 06:30 10th July 2015. The grey and white bars represent night and day respectively, and the black bars at the base of each plot represent the temperature differential between the T_a and T_{core} .

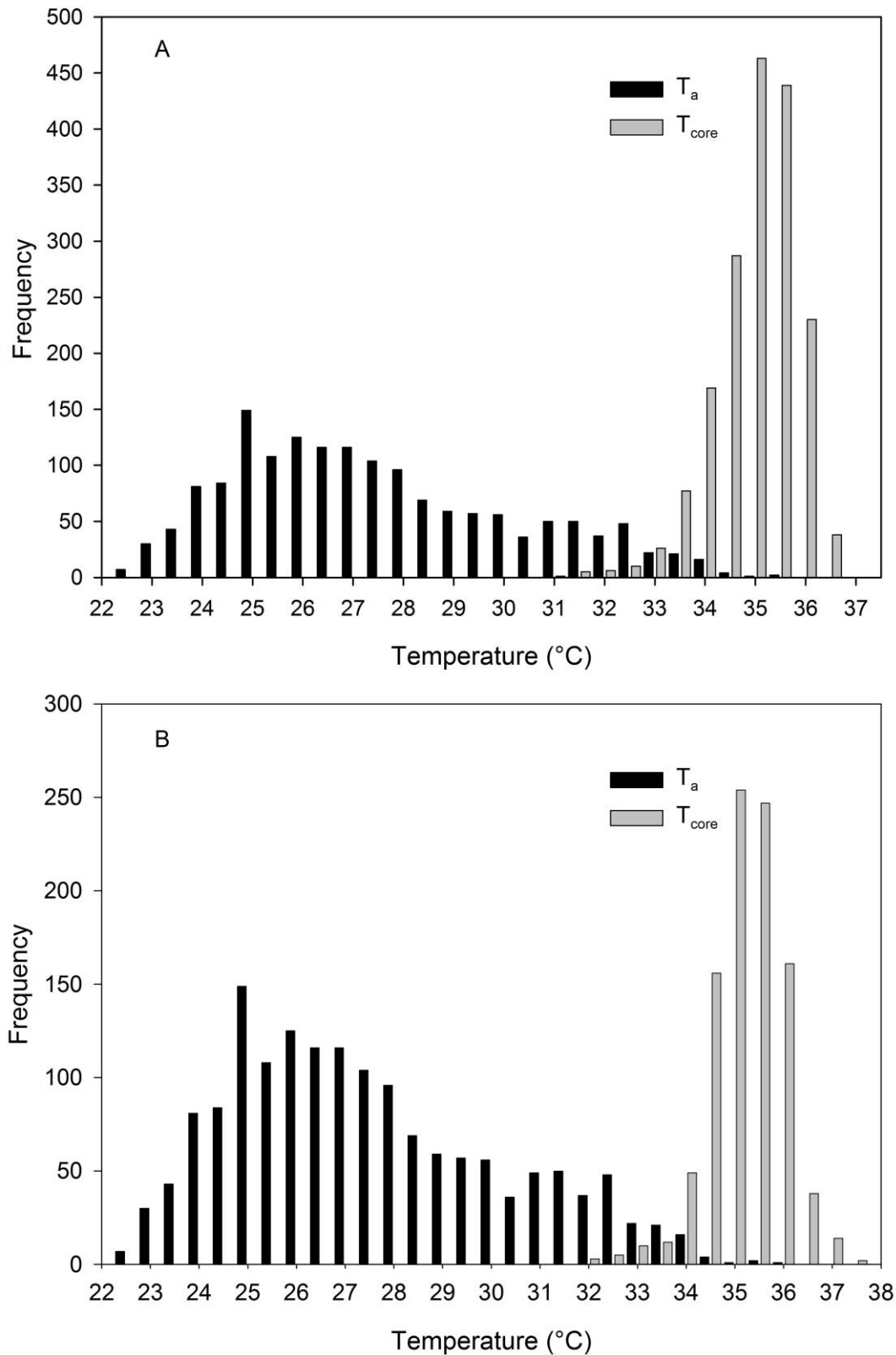


Figure 2-3: The frequency distribution of the ambient temperatures (T_a) and core temperatures (T_{core}) recorded in free-ranging western tarsiers (*Cephalopachus bancanus*). A) The distribution of temperatures corresponding to the female tarsier in Figure 2-2A. B) The distribution of temperatures corresponding to the male tarsier in Figure 2-2B.

Table 2-3: The daily mean, maximum and minimum ambient (T_a) and tarsier core (T_{core}) temperatures observed throughout the period of free-ranging data collection (values in parenthesis represent the range in daily means), as well as the times at which these parameters were most frequently observed. Photophase corresponds to the animals rest phase, whereas scotophase corresponds to their active phase. ♂ = male, ♀ = female, max = highest temperature, min = lowest temperature, n = periods of observations and † indicates a significant ($p < 0.05$) Rayleigh's test.

	Photophase (p-phase)			Scotophase (α-phase)		
	T_a (n = 33)	♀ T_{core} (n = 33)	♂ T_{core} (n = 16)	T_a (n = 33)	♀ T_{core} (n = 33)	♂ T_{core} (n = 17)
Mean	28.6°C (25.75-31.0°C)	34.4°C (32.9-35.2°C)	34.7°C (33.9-35.6°C)	25.8°C (23.8-27.7°C)	35.1°C (33.5-35.7°C)	35.3°C (34.1-36.4°C)
Maximum	32.1°C (28.0-35.2°C)	35.8°C (34.9-36.8°C)	35.7°C (35.0-36.9°C)	28.2°C (24.3-29.9°C)	35.8°C (34.0-36.4°C)	36.1°C (37.1-35.3°C)
Minimum	24.3°C (22.3-27.0°C)	33.4°C (31.5-34.4°C)	33.7°C (31.6-35.3°C)	24.3°C (22.2-27.1°C)	34.4°C (30.6-35.3°C)	34.4°C (32.2-35.8°C)
The time of the day at which maximum and minimum temperatures occurred and the relative percentage of observations						
	T_a max	T_a min	♀ T_{core} max	♀ T_{core} min	♂ T_{core} max	♂ T_{core} min
Photophase (p-phase)	† 13:00 (12%); 13:30 (15%); 14:00 (24%); 14:30 (18 %)	† 06:30 (15%); 07:00 (61%)	† 06:30 (76%)	† 08:30 (15%); 09:00 (21%); 09:30 (30%)	06:30 (53%); 14:30 (12%); 16:00 (18%)	† 08:30 (12%); 09:00 (24%); 09:30 (24%); 10:00 (12%); 18:00 (12%)
Scotophase (α-phase)	† 00:30 (12%); 18:30 (82%);	† 05:30 (24%); 06:00 (45%)	† 06:00 (18%); 19:00 (42%)	† 02:00 (12%); 04:00 (12%); 04:30 (15%); 18:30 (27%);	06:00 (18%); 19:00 (59%)	† 00:00 (18%); 00:30 (12%); 01:00 (12%); 03:30 (18%);

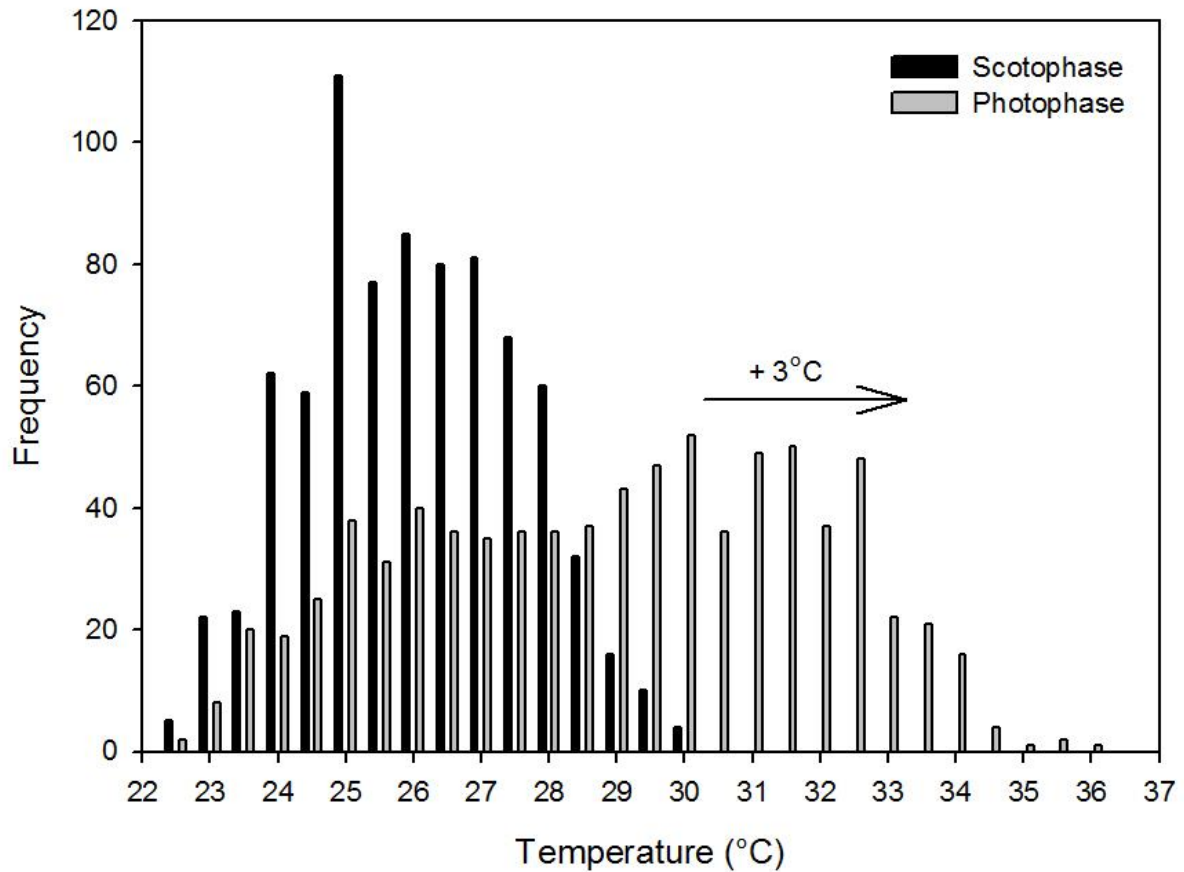


Figure 2-4: The frequency distribution of the scotophase and photophase ambient temperatures recorded within the forest at Sama Jaya Nature Reserve from 00:00 24th June 2015 to 00:00 27th July 2015. The arrow indicates the commonly predicted shift of three degree Celsius in the modal temperature associated with global warming.

Discussion

Our study provides no clear indication of metabolic suppression or the characteristic concomitant decrease in body temperature associated with adaptive heterothermy in either wild-caught animals measured in the laboratory or in free-ranging individuals (see Geiser, 2004). For the field data, having a single individual per sex limits our deductions regarding the finer details and interpretation of the species' free-ranging T_{core} pattern. Nonetheless, we are able to provide a few key observations and relate them to data from the laboratory.

The lowest temperatures to which tarsiers were exposed, both in the laboratory and in the field, was approximately 20°C, that is, about 5°C lower than the T_{lc} . Typically, it is at temperatures $< T_{lc}$ that heterotherms enter daily torpor or hibernation. Instead, tarsiers appeared to show cold sensitivity. This is because during laboratory measurements, it became increasingly difficult for the animals to maintain $T_{bs} > 33^{\circ}\text{C}$ at $T_{as} < 30^{\circ}\text{C}$ despite their thermoregulatory attempts to increase metabolic heat production. The decreasing trend in T_b at low temperatures suggests that the animals may have been heading towards mild pathological hypothermia at the lowest ambient temperatures. One observation in the free-ranging data provides a margin of support for our argument. T_{core} in the female decreased to 31.5°C during the afternoon on the 29th of June (Fig. 2-2A) following a cold snap and only rebounded after T_a started to increase later during the day. However, the male did not display the same response during the cold snap. That tarsiers may be acutely cold sensitive does not seem farfetched considering that they evolved in a warm tropical climate and have never left those conditions (Jablonski, 2003; Simons, 2003).

In contrast to the body temperature profile observed in free-ranging *T. syrichta* individuals on Bohol Island in the Philippines (Lovegrove et al., 2014a), free-ranging *C. bancanus* maintained higher T_{cores} during their active phase. In that study, *T. syrichta* displayed an extremely unusual pattern for a nocturnal mammal (Aschoff, 1983; Refinetti and Menaker, 1992) with consistent and considerable heat storage occurring during the photophase. Heat storage, barring one exceptionally hot day where T_{core} was elevated to $T_{core} = 36.8^{\circ}\text{C}$ (see July 13, Fig. 2-2B), did not occur routinely during the photophase in this study. Furthermore, *C. bancanus* maintained a range of T_{cores} that bordered the boundary between basoendothermy and “mesoendothermy” ($35^{\circ}\text{C} \leq T_b \leq 37.9^{\circ}\text{C}$) (*sensu* Lovegrove, 2012a), but were more frequently representative of mesoendothermic values. Thus, whereas *T. syrichta* appeared to be strict basoendotherms (Lovegrove et al., 2014a), *C. bancanus* maintained normothermic T_{cores} which were closer to the average T_b of the primate clade (see Clarke et al., 2010). The free-ranging T_{core} in our study suggests that tarsiers are intermediate between the ancestral low T_b condition and the derived higher T_b condition (Lovegrove, 2012a), which

supports the phylogenetic position of tarsiers as the link between the older and derived primates (Hartig et al., 2013). Notably, the data from *T. syrichta* were skin temperatures (T_{skin}) measured as a proxy for core temperatures; a technique which is susceptible to unreliable measures (Dausmann, 2012; Lovegrove et al., 2014a) especially, as we suspect is the case in the Bohol study, by loose-fitting collars. For now, heat storage may not be as prominent in tarsiers as the Bohol data suggested, but this may change within the near future.

It has been argued that the temperature increase associated with global warming will have a mild effect on *T. syrichta*, in spite of their T_{skin} pattern, because their TNZ was above the maximum T_{as} observed in their habitat (McNab and Wright, 1987; Lovegrove et al., 2014a). The same notion does not apply here. The TNZ range of *C. bancanus* (Fig. 2-1A), is approximately 5°C lower than that of *T. syrichta*. A conservative prediction of a 3°C increase in T_{as} , relative to the modal T_{as} observed in our study, would result in a large proportion of the photophase T_{as} shifting above the TNZ of *C. bancanus* (see Fig. 2-4). Offloading excess body heat as T_{a} approaches T_{core} is only effective through evaporative cooling (Sherwood and Huber, 2010). The laboratory data show that heat dissipation through evaporation, in a dry atmosphere, was only effective up to $T_{\text{a}} = 30^{\circ}\text{C}$ (Fig. 2-1C-D). Above that, heat storage culminated in dangerously high T_{subS} (Fig. 2-1B). Although some mammals survive at $T_{\text{b}} > 40^{\circ}\text{C}$ (Prothero, 2015), endotherms, in general, seemingly only survive increases in T_{b} of up to 6°C relative to their normothermic T_{b} (Schmidt-Nielsen, 1997). Thus, given that a thermal gradient between an animal's core and skin is required to facilitate heat loss (Sherwood and Huber, 2010) and that *C. bancanus* maintain a normothermic T_{b} of ca. 35°C, T_{subS} approaching 38°C suggests that significant heat storage is occurring and that T_{core} is likely higher. Worrisomely, wild tarsiers are faced with constantly high relative humidity conditions which retard heat dissipation. A further concern is the argument that the rate of global warming has been greatly underestimated by the IPCC (IPCC, 2007; Rahmstorf et al., 2007; van Oldenborgh et al., 2009), and might be much higher than the feasible adaptive response of the species (Hughes, 2000; Root et al., 2003; Quintero and Wiens, 2013). Increases in the severity and frequency of extreme weather events, which are known to have devastating consequences (Boyles et al., 2011), have also been predicted (Meehl and Tebaldi, 2004; Jentsch et al., 2007; Luber and McGehehin, 2008). The future survival of wild *C. bancanus* is therefore highly questionable and supports the concerns expressed by Lovegrove et al. (2014a).

We conclude, for the moment, that adaptive heterothermy has yet to be observed in tarsiers. The apparent loss of adaptive heterothermy in tarsiers, as well as the lack of irrefutable evidence for adaptive heterothermy in other haplorrhines, suggests that adaptive heterothermy may have been lost either in the ancestor of the tarsiers or in the tarsier clade.

We muse on our rationale below and we identify also several potential haplorrhine primates species in which a search for adaptive heterothermy might fruitfully aid in resolving the primate adaptive heterothermy phenotype.

Barring a few notable exceptions within the carnivores, monotremes and rodents, adaptive heterothermy is mostly observed in mammals smaller than 1 kg.; mean weight of approximately 340g (Geiser, 1998; Lovegrove, 2012a; Ruf and Geiser, 2015). Body size is an important consideration because thermal inertia in large-bodied animals hampers the reduction in T_b and thus the energetic benefits of adaptive heterothermy (Geiser, 1998; Ruf and Geiser, 2015). Observations of adaptive heterothermy in large and small sized animals suggests that they may represent different conditions but more work on this topic is required (Geiser, 2001). Specifically, within primates, heterothermic species range in M_b from the 30g Madame Berthe's mouse lemur (*Microcebus berthae*; Dausmann and Warnecke, 2016) to the 350g furry-eared dwarf lemur (*Cheirogaleus crossleyi*; Blanco and Rahalinarivo, 2010; Blanco et al., 2013), but the heaviest known primate heterotherm is the 400g pygmy slow loris (*Nycticebus pygmaeus*; Ruf et al., 2015). No adaptive heterothermy was observed in the large 600g sportive lemur (*Lepilemur ruicaudatus*; Schmid and Ganzhorn, 1996). Thus, even though body size may not be the only determining factor, it is an important consideration, so we analysed the database of Isler et al. (2008) to identify other potential primate candidates that, based on body mass, could be heterotherms. The database contained the body mass values for 239 primates, 177 of which were haplorrhines. Excluding the tarsiers (Tarsiidae), there are 11 other haplorrhines that weigh 500g or less and all belong to the family Cebidae namely *Callithrix pygmaea*, *C. jacchus*, *C. penicillate*, *C. argentata*, *C. humeralifera*, *C. aurita*, *Saguinus fuscicollis*, *S. niger*, *S. oedipus*, *S. nigricollis* and *Callimico goeldii*. To the best of our knowledge, no evidence of torpor has been observed in these primates. However, although metabolic studies do exist for some (Boere et al., 2005; Kuehnel et al., 2012; Go et al., 2015; Ross et al., 2015; Lelegren et al., 2016), thermoregulatory studies with the potential to observe adaptive heterothermy have only been conducted for *C. jacchus* (Petry et al., 1986), *C. pygmaea* (Genoud et al., 1997; Tattersall, 2012), *S. oedipus* (Stonerook et al., 1994) and *C. goeldii* (Kälin et al., 2003; Power et al., 2003). While we cannot, at this point, discount the possibility of torpor in all Platyrrhini, the lack of torpor by *C. pygmaea*, the smallest and most eligible platyrrhine heterotherm, is congruent with our reasoning. We urge further investigation and suggest that future studies focus on the species listed here as they are the most likely to disprove our idea of the loss of adaptive heterothermy in non-Shrepsirrhini.

The reason(s) why adaptive heterothermy appears to have been lost in tarsiers at the Strepsirrhini-Haplorrhini split is unclear. However, it is worth exploring the similarities and dissimilarities between heterothermic members of the Strepsirrhini and members of the

Tarsiidae which might also explain the apparent loss of adaptive heterothermy in other haplorrhines. We share thoughts on factors directly related to energy expenditure namely, body size including the size of the brain, nocturnal habits, the effect of diet, reproduction and locomotion.

As discussed earlier, based on their M_b , tarsiers should be eligible to express adaptive heterothermy. Thus, there may be another size-related factor that accounts for the lack of torpor use, such as brain size. Brain tissue is a metabolically expensive tissue. The increase in brain size of the haplorrhines and the consequent increase in metabolic demands reflected in higher BMRs (Isler and van Schaik, 2006) may have prohibited the use of adaptive heterothermy (Lovegrove, 2017). Tarsiers are nocturnal hunters that leap from tree to tree. The ability to successfully navigate, let alone hunt, in low light environments requires tremendous visual acuity, depth perception and neuronal accompaniment (Collins et al., 2005). Indeed, tarsiers display numerous specialized adaptations such as an enlarged lens, cornea and retina, as well as a high density of rods in the retina ($> 300,000$ per mm^2) in addition to neuronal adjustments, most notably enlarged primary and secondary visual cortex regions (Castenholz, 1984; Collins et al., 2005). However, these adaptations appear subject to a trade-off. Tarsier brains, relative to other primates, do not show an increase in mass, endocranial volume or encephalization quotient (Stephan, 1984; Grabowski et al., 2016). Instead, tarsiers compensate through a morphological readjustment of the brain by having enlarged occipital and temporal lobes and a diminished frontal lobe (Schwartz, 2003).

The Expensive-Tissue Hypothesis predicts that the metabolic costs associated with large brains in primates are off-set by reductions in the equally costly splanchnic tissue (Aiello and Wheeler, 1995). Thus, in addition to the morphological readjustment in tarsier brains, a further trade-off may be their dietary simplification and specialization. As previously mentioned, tarsiers are the only strict carnivorous primates, and it has been suggested that their dietary constriction may preclude the ability to employ adaptive heterothermy (Dausmann, 2008). However, the restricting factor may not be their diet *per se*, but rather the trade-off which their diet represents. An expansion of The Expensive-Tissue Hypothesis, the Expensive Brain Framework Hypothesis (Isler and van Schaik, 2009), proposed that the costs associated with relatively large brains must either accompany an increase in energy turnover or a reduction in energy expenditure; not limited to digestion. Within this framework, a portion of the energetic costs associated with a large primate brain and visual adaptations needed for their carnivorous nocturnal lifestyle (Crompton and Andau, 1986; 1987; Jablonski and Crompton, 1994) may be shared by a reduction in foetal growth rate, reproductive output and the cost of locomotion.

Tarsiers typically produce a single offspring and have gestation periods ranging between 157-180 days, longer than the gestation period of *Microcebus* spp. and *Cheirogaleus* spp., but comparable to those of *Galago* spp. and *Loris* spp. (Izard et al., 1985; Roberts, 1994). However, the M_b of tarsier neonates relative to their adult M_b as well as their neonate brain size is significantly larger than other primates (Roberts, 1994). The large neonatal brain size explains why, despite similar or even slower postnatal growth rates and weaning periods relative to other primates, tarsier infants have extremely rapid behavioural development, particularly in foraging behaviour (Roberts, 1994). As such, development factors, in combination with dietary constraints may preclude adaptive heterothermy. Furthermore, in *C. bancanus* at least, homeothermy may be related to the fact that they remain reproductively active throughout the year (MacKinnon and MacKinnon, 1980; Wright et al., 1986). Males thus continuously produce sperm and a greater variation in body temperature may compromise sperm viability as spermatogenesis, sperm storage and sperm maturation processes are optimized at 34-36°C (Lovegrove, 2014).

With regards to the cost of locomotion, irrespective of the mode of locomotion the energetic investment is proportional to M_b (Schmidt-Nielsen, 1997; Withers et al., 2016). Of the various modes of locomotion, saltation, while at low speed, is known to be energetically more costly than typical walking. However, it becomes energetically more efficient as the speed increases (Withers et al., 2016). Tarsiers do not typically hop around on the ground at low speed, but leap fleetly around in trees. In comparison to other saltatorial primates, tarsiers seemingly have the lowest energetic investment (Warren and Crompton, 1998). Warren and Crompton (1998) considered the kinetic cost of leaping, M_b , distant travelled, home range size and metabolism, and showed that even though tarsiers travelled the farthest and had the largest home range of all their study species', they had the lowest absolute and relative cost of locomotion. This reduction in energetic expense presumably stems from the tarsiers' musculo-skeletal anatomical adaptations (Jouffroy et al., 1984; Peters and Preuschoft, 1984; Schultz, 1984; Anemone and Nachman, 2003); the most obvious of which is the eponymous elongated tarsal bones.

Conflict of interest statement

The authors declare no financial, or other, competing interests.

Author contributions

S.W. and B.G.L. conceived and designed the study. S.W. performed the data collection, analyses and drafted the manuscript. B.G.L. contributed to and approved the manuscript.

A.A.T. secured capture permits, provided logistical support, as well as approved the manuscript.

Funding

The research was support by the National Research Foundation (South Africa), GreenMatter fellowship (South Africa) and their partners, as well as the Ministry of Higher education of Malaysia (FRGS/1/2013/ST03/UNIMAS/01/2).

Acknowledgements

We thank the friendly staff at Sama Jaya Nature Reserve, as well as Dr. Samuel Kiyui and his staff at Country Veterinary Clinic. We also thank Danielle Levesque, Tunmise Otitoju, Miyn Naharuddin, Anthony Pine, Sally Soo and Frances Hii for their help in the field.

References

- Aiello, L.C., and Wheeler, P. (1995). The Expensive-Tissue Hypothesis: The brain and the digestive system in human and primate evolution. *Curr. Anthropol.* 36(2), 199-221. doi: <http://www.jstor.org/stable/2744104>.
- Anemone, R.L., and Nachman, B.A. (2003). "Morphometrics, functional anatomy, and the biomechanics of locomotion among tarsiers," in *Tarsiers: Past, Present, and Future*, eds. P.C. Wright, E.L. Simons & S. Gursky. (New Brunswick Rutgers University Press), 97-120.
- Aschoff, J. (1983). Circadian control of body temperature. *J. Therm. Biol.* 8(1), 143-147. doi: 10.1016/0306-4565(83)90094-3.
- Augee, M.L., and Gooden, B.A. (1992). "Monotreme hibernation—some afterthoughts," in *Platypus and Echidnas* ed. M.L. Augee. (Sydney: Royal Zoological Society of New South Wales), 174-176.
- Aujard, F., Perret, M., and Vannier, G. (1998). Thermoregulatory responses to variations of photoperiod and ambient temperature in the male lesser mouse lemur: a primitive or an advanced adaptive character? *J. Comp. Physiol. B.* 168(7), 540-548. doi: 10.1007/s003600050175.
- Bennett, A.F. (1991). The evolution of activity capacity. *J. Exp. Biol.* 160, 1-23.
- Bennett, A.F., and Ruben, J.A. (1979). Endothermy and activity in vertebrates. *Science* 206(4419), 649-654. doi: 10.1126/science.493968.

- Bieber, C., Lebl, K., Stalder, G., Geiser, F., and Ruf, T. (2014). Body mass dependent use of hibernation: why not prolong the active season, if they can? *Funct. Ecol.* 28(1), 167-177. doi: 10.1111/1365-2435.12173.
- Bieber, C., and Ruf, T. (2009). Summer dormancy in edible dormice (*Glis glis*) without energetic constraints. *Naturwissenschaften* 96(1), 165-171. doi: 10.1007/s00114-008-0471-z.
- Blanco, M.B., Dausmann, K.H., Ranaivoarisoa, J.F., and Yoder, A.D. (2013). Underground hibernation in a primate. *Sci. Rep.* 3, 1768. doi: 10.1038/srep01768.
- Blanco, M.B., and Rahalinarivo, V. (2010). First direct evidence of hibernation in an eastern dwarf lemur species (*Cheirogaleus crossleyi*) from the high-altitude forest of Tsinjoarivo, central-eastern Madagascar. *Naturwissenschaften* 97(10), 945-950. doi: 10.1007/s00114-010-0707-6.
- Boere, V., Pinheiro, E.C., de Oliveira e Silva, I., Paludo, G.R., Canale, G., Pianta, T., et al. (2005). Comparison between sex and age class on some physiological, thermal, and hematological indices of the cerrado's marmoset (*Callithrix penicillata*). *J. Med. Primatol.* 34(3), 156-162.
- Boyles, J.G., Seebacher, F., Smit, B., and McKechnie, A.E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* 51, 676-690.
- Burnham, K.P., and Anderson, D.R. (2003). *Model selection and multimodel inference: a practical information-theoretic approach*. New York: Springer.
- Castenholz, A. (1984). "The eye of *Tarsius*," in *Biology of tarsiers*, ed. C. Niemitz. (Stuttgart: Gustav Fischer Verlag), 303-318.
- Clarke, A., and Pörtner, H.O. (2010). Temperature, metabolic power and the evolution of endothermy. *Biol. Rev.* 85(4), 703-727. doi: 10.1111/j.1469-185X.2010.00122.x.
- Clarke, A., Rothery, P., and Isaac, N.J.B. (2010). Scaling of basal metabolic rate with body mass and temperature in mammals. *J. Anim. Ecol.* 79, 610-619. doi: 10.1111/j.1365-2656.2010.01672.x.
- Clarke, R.W. (1943). The respiratory exchange of *Tarsius spectrum*. *J. Mammal.* 24(1), 94-96. doi: 10.2307/1374785.
- Collins, C.E., Hendrickson, A., and Kaas, J.H. (2005). Overview of the visual system of tarsius. *Anat. Rec. A. Discov. Mol. Cell. Evol. Biol.* 287A(1), 1013-1025. doi: 10.1002/ar.a.20263.

- Crompton, A.W., Taylor, C.R., and Jagger, J.A. (1978). Evolution of homeothermy in mammals. *Nature* 272, 333-336.
- Crompton, R.H., and Andau, P.M. (1986). Locomotion and habitat utilization in free-ranging *Tarsius bancanus*: a preliminary report. *Primates* 27(3), 337-355. doi: 10.1007/BF02382075.
- Crompton, R.H., and Andau, P.M. (1987). Ranging, activity rhythms, and sociality in free-ranging *Tarsius bancanus*: a preliminary report. *Int. J. Primatol.* 8(1), 43-71. doi: 10.1007/BF02737113.
- Dausmann, K.H. (2008). "Hypometabolism in primates: torpor and hibernation," in *Hypometabolism in animals: hibernation, torpor and cryobiology*, eds. B.G. Lovegrove & A.E. McKechnie. (University of KwaZulu-Natal, Pietermaritzburg: 13th International Hibernation Symposium), 327-336.
- Dausmann, K.H. (2012). The pitfalls of body temperature measurements. *Naturwissenschaften* 99(6), 511-513. doi: 10.1007/s00114-012-0924-2.
- Dausmann, K.H. (2014). Flexible patterns in energy savings: heterothermy in primates. *J. Zool.* 292(2), 101-111. doi: 10.1111/jzo.12104.
- Dausmann, K.H., Ganzhorn, J.U., and Heldmaier, G. (2000). "Body temperature and metabolic rate of a hibernating primate in Madagascar: preliminary results from a field study," in *Life in the Cold: Eleventh International Hibernation Symposium*, eds. G. Heldmaier & M. Klingenspor. (Berlin, Heidelberg: Springer Berlin Heidelberg), 41-47.
- Dausmann, K.H., Glos, J., Ganzhorn, J.U., and Heldmaier, G. (2005). Hibernation in the tropics: lessons from a primate. *J. Comp. Physiol. B.* 175(3), 147-155. doi: 10.1007/s00360-004-0470-0.
- Dausmann, K.H., and Warnecke, L. (2016). Primate torpor expression: ghost of the climatic past. *Physiology* 31(6), 398-408. doi: 10.1152/physiol.00050.2015.
- Farmer, C.G. (2000). Parental care: the key to understanding endothermy and other convergent features in birds and mammals. *Am. Nat.* 155, 326-334. doi: 10.1086/303323.
- Geiser, F. (1998). Evolution of daily torpor and hibernation in birds and mammals: importance of body size. *Clin. Exp. Pharmacol. Physiol.* 25(9), 736-740. doi: 10.1111/j.1440-1681.1998.tb02287.x.
- Geiser, F. (2001). "Hibernation: endotherms," in *eLS*. (Chichester: John Wiley & Sons, Ltd).

- Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu. Rev. Physiol.* 66, 239-274. doi: 10.1146/annurev.physiol.66.032102.115105.
- Geiser, F., and Brigham, R.M. (2012). "The other functions of torpor," in *Living in a seasonal world: thermoregulatory and metabolic adaptations*, eds. T. Ruf, C. Bieber, W. Arnold & E. Millesi. (Heidelberg, Berlin: Springer), 109-121.
- Geiser, F., and Turbill, C. (2009). Hibernation and daily torpor minimize mammalian extinctions. *Naturwissenschaften* 96(10), 1235-1240. doi: 10.1007/s00114-009-0583-0.
- Genoud, M., Martin, R.D., and Glaser, D. (1997). Rate of metabolism in the smallest simian primate, the pygmy marmoset (*Cebuella pygmaea*). *Am. J. Primatol.* 41(3), 229-245.
- Go, Y.-M., Liang, Y., Uppal, K., Soltow, Q.A., Promislow, D.E.L., Wachtman, L.M., et al. (2015). Metabolic characterization of the common marmoset (*Callithrix jacchus*). *PLOS ONE* 10(11), e0142916. doi: 10.1371/journal.pone.0142916.
- Grabowski, M., Voje, K.L., and Hansen, T.F. (2016). Evolutionary modeling and correcting for observation error support a 3/5 brain-body allometry for primates. *J. Hum. Evol.* 94, 106-116. doi: 10.1016/j.jhevol.2016.03.001.
- Grigg, G.C., Beard, L.A., and Augee, M.L. (2004). The evolution of endothermy and its diversity in mammals and birds. *Physiol. Biochem. Zool.* 77(6), 982-997. doi: 10.1086/425188.
- Groves, C., and Shekelle, M. (2010). The genera and species of Tarsiidae. *Int. J. Primatol.* 31(6), 1071-1082. doi: 10.1007/s10764-010-9443-1.
- Gursky, S. (2000). Effect of seasonality on the behavior of an insectivorous primate, *Tarsius spectrum*. *Int. J. Primatol.* 21(3), 477-495. doi: 10.1023/a:1005444020059.
- Hansen, T.F. (1997). Stabilizing selection and the comparative analysis of adaptation. *Evolution* 51(5), 1341-1351. doi: 10.2307/2411186.
- Hartig, G., Churakov, G., Warren, W.C., Brosius, J., Makalowski, W., and Schmitz, J. (2013). Retrophylogenomics place tarsiers on the evolutionary branch of anthropoids. *Sci. Rep.* 3:1756. doi: 10.1038/srep01756.
- Hayes, J.P., and Garland Jr, T. (1995). The evolution of endothermy: testing the aerobic capacity model. *Evolution* 49(5), 836-847. doi: 10.2307/2410407.
- Hughes, L. (2000). Biological consequences of global warming: is the signal already apparent? *Trends Ecol. Evol.* 15(2), 56-61. doi: 10.1016/s0169-5347(99)01764-4.

- IPCC (2007). *Climate Change 2007: Synthesis Report. Contribution of working groups I, II and III to the fourth assessment report of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland.
- Isler, K., Christopher Kirk, E., Miller, J.M.A., Albrecht, G.A., Gelvin, B.R., and Martin, R.D. (2008). Endocranial volumes of primate species: scaling analyses using a comprehensive and reliable data set. *J. Hum. Evol.* 55(6), 967-978. doi: <http://dx.doi.org/10.1016/j.jhevol.2008.08.004>.
- Isler, K., and van Schaik, C.P. (2006). Metabolic costs of brain size evolution. *Biol. Lett.* 2(4), 557-560. doi: 10.1098/rsbl.2006.0538.
- Isler, K., and van Schaik, C.P. (2009). The Expensive Brain: A framework for explaining evolutionary changes in brain size. *J. Hum. Evol.* 57(4), 392-400. doi: 10.1016/j.jhevol.2009.04.009.
- Izard, M.K., Wright, P.C., and Simons, E.L. (1985). Gestation length in *Tarsius bancanus*. *Am. J. Primatol.* 9(4), 327-331. doi: 10.1002/ajp.1350090408.
- Jablonski, N.G. (2003). "The evolution of the Tarsiid niche," in *Tarsiers: past, present, and future*, eds. P.C. Wright, E.L. Simons & S. Gursky. (New Brunswick: Rutgers University Press), 35-49.
- Jablonski, N.G., and Crompton, R.H. (1994). Feeding-behavior, mastication, and tooth wear in the Western tarsier (*Tarsius bancanus*). *Int. J. Primatol.* 15(1), 29-59. doi: 10.1007/bf02735233.
- Jentsch, A., Kreyling, J., and Beierkuhnlein, C. (2007). A new generation of climate-change experiments: events, not trends. *Front. Ecol. Environ.* 5(7), 365-374. doi: <http://www.jstor.org/stable/20440702>.
- Jouffroy, F.K., Berge, C., and Niemitz, C. (1984). "Comparative study of the lower extremity in the genus *Tarsius*," in *Biology of tarsiers*, ed. C. Niemitz. (Stuttgart: Gustav Fischer Verlag), 167-190.
- Kälin, N., Martin, R.D., and Genoud, M. (2003). Basal rate of metabolism and temperature regulation in Goeldi's monkey (*Callimico goeldii*). *Comp. Biochem. Phys. A.* 135(2), 279-290. doi: [http://dx.doi.org/10.1016/S1095-6433\(03\)00077-1](http://dx.doi.org/10.1016/S1095-6433(03)00077-1).
- Kemp, T.S. (2006). The origin of mammalian endothermy: a paradigm for the evolution of complex biological structure. *Zool. J. Linnean. Soc.* 147(4), 473-488. doi: 10.1111/j.1096-3642.2006.00226.x.

- Knox, C.M., and Wright, P.G. (1989). Thermoregulation and energy metabolism in the lesser bushbaby, *Galago senegalensis moholi*. *S. Afr. J. Zool.* 24(2), 89-94. doi: 10.1080/02541858.1989.11448138.
- Kobbe, S., and Dausmann, K.H. (2009). Hibernation in Malagasy mouse lemurs as a strategy to counter environmental challenge. *Naturwissenschaften* 96(10), 1221-1227. doi: 10.1007/s00114-009-0580-3.
- Koteja, P. (2000). Energy assimilation, parental care and the evolution of endothermy. *Proc. R. Soc. B.* 267, 479-484. doi: 10.1098/rspb.2000.1025.
- Kuehnel, F., Grohmann, J., Buchwald, U., Koeller, G., Teupser, D., and Einspanier, A. (2012). Parameters of haematology, clinical chemistry and lipid metabolism in the common marmoset and alterations under stress conditions. *J. Med. Primatol.* 41(4), 241-250. doi: 10.1111/j.1600-0684.2012.00550.x.
- Lelegren, M., Liu, Y., Ross, C., Tardif, S., and Salmon, A.B. (2016). Pharmaceutical inhibition of mTOR in the common marmoset: effect of rapamycin on regulators of proteostasis in a non-human primate. *Pathobiol. Aging Age Relat. Dis.* 6, 31793. doi: 10.3402/pba.v6.31793.
- Levesque, D.L., Lobban, K.D., and Lovegrove, B.G. (2014). Effects of reproductive status and high ambient temperatures on the body temperature of a free-ranging basoendotherm. *J. Comp. Physiol. B.* 184(8), 1041-1053. doi: 10.1007/s00360-014-0858-4.
- Lighton, J.R.B. (2008). *Measuring metabolic rates: a manual for scientists*. USA: Oxford University Press.
- Lobban, K.D., Lovegrove, B.G., and Rakotondravony, D. (2014). The energetics of a Malagasy rodent, *Macrotarsomys ingens* (Nesomyinae): a test of island and zoogeographical effects on metabolism. *J. Comp. Physiol. B.* 184(8), 1077-1089. doi: 10.1007/s00360-014-0853-9.
- Lovegrove, B.G. (2000). "Daily heterothermy in mammals: coping with unpredictable environments," in *Life in the cold: eleventh international hibernation symposium*, eds. G. Heldmaier & M. Klingenspor. (Heidelberg, Berlin: Springer), 29-40.
- Lovegrove, B.G. (2012a). The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol. Rev.* 87(1), 128-162. doi: 10.1111/j.1469-185X.2011.00188.x.

- Lovegrove, B.G. (2012b). The evolution of mammalian body temperature: the Cenozoic supraendothermic pulses. *J. Comp. Physiol. B.* 182(4), 579-589. doi: 10.1007/s00360-011-0642-7.
- Lovegrove, B.G. (2014). Cool sperm: why some placental mammals have a scrotum. *J. Evol. Biol.* 27(5), 801-814. doi: 10.1111/jeb.12373.
- Lovegrove, B.G. (2017). A phenology of the evolution of endothermy in birds and mammals. *Biol. Rev.* 92, 1213-1240. doi: 10.1111/brv.12280.
- Lovegrove, B.G., Canale, C., Levesque, D.L., Fluch, G., Rehakova-Petru, M., and Ruf, T. (2014a). Are tropical small mammals physiologically vulnerable to arrhenius effects and climate change? *Physiol. Biochem. Zool.* 87(1), 30-45. doi: 10.1086/673313.
- Lovegrove, B.G., Lobban, K.D., and Levesque, D.L. (2014b). Mammal survival at the Cretaceous–Palaeogene boundary: metabolic homeostasis in prolonged tropical hibernation in tenrecs. *Proc. R. Soc. B.* 281:20141304. doi: 10.1098/rspb.2014.1304.
- Luber, G., and McGeehin, M. (2008). Climate change and extreme heat events. *Am. J. Prev. Med.* 35(5), 429-435. doi: 10.1016/j.amepre.2008.08.021.
- MacKinnon, J., and MacKinnon, K. (1980). The behavior of wild spectral tarsiers. *Int. J. Primatol.* 1(4), 361-379. doi: 10.1007/bf02692280.
- Matsui, A., Rakotondraparany, F., Munechika, I., Hasegawa, M., and Horai, S. (2009). Molecular phylogeny and evolution of prosimians based on complete sequences of mitochondrial DNAs. *Gene* 441(1–2), 53-66. doi: 10.1016/j.gene.2008.08.024.
- Mazerolle, M.J. (2015). "AICcmodavg: model selection and multimodel inference based on (Q)AIC(c)". R package version 2.0-3 ed.).
- McNab, B.K. (1978). The evolution of endothermy in the phylogeny of mammals. *Am. Nat.* 112(983), 1-21. doi: <http://www.jstor.org/stable/2460134>.
- McNab, B.K., and Wright, P.C. (1987). Temperature regulation and oxygen consumption in the Philippine tarsier *Tarsius syrichta*. *Physiol. Zool.* 60(5), 596-600. doi: 10.2307/30156133.
- Meehl, G.A., and Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* 305(5686), 994-997. doi: 10.1126/science.1098704.
- Meireles, C.M., Czelusniak, J., Page, S.L., Wildman, D.E., and Goodman, M. (2003). "Phylogenetic position of tarsiers within the order primates: Evidence from gamma-

- globin DNA sequences," in *Tarsiers: past, present, and future*, eds. P.C. Wright, E.L. Simons & S. Gursky. (New Brunswick: Rutgers University Press), 145-160.
- Muggeo, V.M.R. (2008). Segmented: an R package to fit regression models with broken-line relationships. *R news* 8(1), 20-25.
- Mzilikazi, N., Masters, J.C., and Lovegrove, B.G. (2006). Lack of torpor in free-ranging southern lesser galagos, *Galago moholi*: ecological and physiological considerations. *Folia Primatol.* 77(6), 465-476. doi: 10.1159/000095392.
- Neri-Arboleda, I., Stott, P., and Arboleda, N.P. (2002). Home ranges, spatial movements and habitat associations of the Philippine tarsier (*Tarsius syrichta*) in Corella, Bohol. *J. Zool.* 257(3), 387-402. doi: 10.1017/S0952836902000997.
- Niemitz, C. (1984). "Synecological relationships and feeding behaviour of the genus *Tarsius*," in *Biology of tarsiers*, ed. C. Niemitz. (Stuttgart: Gustav Fischer Verlag), 59-75.
- Niemitz, C., Klauer, G., and Eins, S. (1984). "The interscapular brown fat body in *Tarsius bancanus*, with comparisons to *Tupaia* and man," in *Biology of tarsiers*, ed. C. Niemitz. (Stuttgart: Gustav Fischer Verlag), 257-273.
- Nowack, J., Cooper, C.E., and Geiser, F. (2016). Cool echidnas survive the fire. *Proc. R. Soc. B.* 283:20160382. doi: 10.1098/rspb.2016.0382.
- Nowack, J., Mzilikazi, N., and Dausmann, K.H. (2010). Torpor on demand: heterothermy in the non-lemur primate *Galago moholi*. *PLOS ONE* 5(5), e10797. doi: 10.1371/journal.pone.0010797.
- Nowack, J., Rojas, A.D., Körtner, G., and Geiser, F. (2015). Snoozing through the storm: torpor use during a natural disaster. *Sci. Rep.* 5, 11243. doi: 10.1038/srep11243.
- Ortmann, S., Heldmaier, G., Schmid, J., and Ganzhorn, J.U. (1997). Spontaneous daily torpor in Malagasy mouse lemurs. *Naturwissenschaften* 84(1), 28-32.
- Perelman, P., Johnson, W.E., Roos, C., Seuánez, H.N., Horvath, J.E., Moreira, M.A.M., et al. (2011). A Molecular Phylogeny of Living Primates. *PLOS Genet.* 7(3), e1001342. doi: 10.1371/journal.pgen.1001342.
- Peters, A., and Preuschoft, H. (1984). "External biomechanics of leaping in *Tarsius* and its morphological and kinematic consequences," in *Biology of tarsiers*, ed. C. Niemitz. (Stuttgart: Gustav Fischer Verlag), 227-255.
- Petry, H., Riehl, I., and Zucker, H. (1986). Energieumsatzmessungen an Weißbüscheläffchen (*Callithrix jacchus*). *J. Anim. Physiol. Anim. Nutr.* 55(1-5), 214-224. doi: 10.1111/j.1439-0396.1986.tb00722.x.

- Pinheiro, J., Bates, D., DebRoy, S., Deepayan, S., and Team, R.C. (2016). "Nlme: linear and nonlinear mixed effects models". R package version 3.1-128.
- Pocock, R.I. (1918). On the external characters of the lemurs and of *Tarsius*. *Proc. Zool. Soc. Lond.* 88(1-2), 19-53. doi: 10.1111/j.1096-3642.1918.tb02076.x.
- Power, M.L., Tardif, S.D., Power, R.A., and Layne, D.G. (2003). Resting energy metabolism of Goeldi's monkey (*Callimico goeldii*) is similar to that of other callitrichids. *Am. J. Primatol.* 60(2), 57-67.
- Prothero, J.W. (2015). *The design of mammals: A scaling approach*. Cambridge, UK: Cambridge University Press.
- Quintero, I., and Wiens, J.J. (2013). Rates of projected climate change dramatically exceed past rates of climatic niche evolution among vertebrate species. *Ecol. Lett.* 16(8), 1095-1103. doi: 10.1111/ele.12144.
- R Core Team (2017). "R: A language and environment for statistical computing". Vienna, Austria: R Foundation for Statistical Computing.
- Rahmstorf, S., Cazenave, A., Church, J.A., Hansen, J.E., Keeling, R.F., Parker, D.E., et al. (2007). Recent climate observations compared to projections. *Science* 316(5825), 709. doi: 10.1126/science.1136843.
- Refinetti, R., and Menaker, M. (1992). The circadian rhythm of body temperature. *Physiol. Behav.* 51(3), 613-637. doi: 10.1016/0031-9384(92)90188-8.
- Roberts, M. (1994). Growth, development, and parental care in the western tarsier (*Tarsius bancanus*) in captivity: Evidence for a "slow" life-history and nonmonogamous mating system. *Int. J. Primatol.* 15(1), 1-28. doi: 10.1007/BF02735232.
- Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C., and Pounds, J.A. (2003). Fingerprints of global warming on wild animals and plants. *Nature* 421(6918), 57-60. doi: 10.1038/nature01333.
- Ross, C., Salmon, A., Strong, R., Fernandez, E., Javors, M., Richardson, A., et al. (2015). Metabolic consequences of long-term rapamycin exposure on common marmoset monkeys (*Callithrix jacchus*). *Aging* 7(11), 964-973.
- Ruf, T., and Geiser, F. (2015). Daily torpor and hibernation in birds and mammals. *Biol. Rev.* 90(3), 891-926. doi: 10.1111/brv.12137.
- Ruf, T., Streicher, U., Stalder, G.L., Nadler, T., and Walzer, C. (2015). Hibernation in the pygmy slow loris (*Nycticebus pygmaeus*): multiday torpor in primates is not restricted to Madagascar. *Sci. Rep.* 5, 17392. doi: 10.1038/srep17392.

- Sa'adi, Z., Shahid, S., Ismail, T., Chung, E.-S., and Wang, X.-J. (2017). Trends analysis of rainfall and rainfall extremes in Sarawak, Malaysia using modified Mann–Kendall test. *Meteorol Atmos Phys*. doi: 10.1007/s00703-017-0564-3.
- Schmid, J. (2000). Daily torpor in the gray mouse lemur (*Microcebus murinus*) in Madagascar: energetic consequences and biological significance. *Oecologia* 123(2), 175-183. doi: 10.2307/4222607.
- Schmid, J., and Ganzhorn, J.U. (1996). Resting metabolic rates of *Lepilemur ruficaudatus*. *Am. J. Primatol.* 38(2), 169-174.
- Schmid, J., and Ganzhorn, J.U. (2009). Optional strategies for reduced metabolism in gray mouse lemurs. *Naturwissenschaften* 96(6), 737-741. doi: 10.1007/s00114-009-0523-z.
- Schmid, J., and Kappeler, P.M. (1998). Fluctuating sexual dimorphism and differential hibernation by sex in a primate, the Gray mouse lemur (*Microcebus murinus*). *Behav. Ecol. Sociobiol.* 43(2), 125-132. doi: <http://www.jstor.org/stable/4601496>.
- Schmidt-Nielsen, K. (1997). *Animal physiology: adaptation and environment*. Cambridge (UK): Cambridge University Press.
- Schmitz, J., Ohme, M., and Zischler, H. (2001). SINE insertions in cladistic analyses and the phylogenetic affiliations of *Tarsius bancanus* to other primates. *Genetics* 157(2), 777-784.
- Schultz, M. (1984). "Osteology and myology of the upper extremity of *Tarsius*," in *Biology of tarsier*, ed. C. Niemitz. (Stuttgart: Gustav Fischer Verlag), 143-165.
- Schwartz, J.H. (1984). "What is a Tarsier?," in *Living Fossils*, eds. N. Eldredge & S.M. Stanley. (New York, NY: Springer New York), 38-49.
- Schwartz, J.H. (2003). "How close are the similarities between *Tarsius* and other primates," in *Tarsiers: past, present, and future*, eds. P.C. Wright, E.L. Simons & S. Gursky. (New Brunswick: Rutgers University Press), 50-96.
- Sherwood, S.C., and Huber, M. (2010). An adaptability limit to climate change due to heat stress. *Proc. Natl. Acad. Sci. U. S. A.* 107(21), 9552-9555. doi: 10.1073/pnas.0913352107.
- Simons, E.L. (2003). "The fossil record of Tarsier evolution," in *Tarsiers: past, present, and future*, eds. P.C. Wright, E.L. Simons & S. Gursky. (New Brunswick: Rutgers University Press), 9-34.

- Stawski, C., and Geiser, F. (2010). Fat and fed: frequent use of summer torpor in a subtropical bat. *Naturwissenschaften* 97(1), 29-35. doi: 10.1007/s00114-009-0606-x.
- Stawski, C., Körtner, G., Nowack, J., and Geiser, F. (2015). The importance of mammalian torpor for survival in a post-fire landscape. *Biol. Lett.* 11(6). doi: 10.1098/rsbl.2015.0134.
- Stephan, A. (1984). "Morphology of the brain in *Tarsius*," in *Biology of tarsiers*, ed. C. Niemitz. (Stuttgart: Gustav Fischer Verlag), 317-344.
- Stonerook, M.J., Weiss, H.S., Rodriguez-M, M.A., Rodríguez-M, J.V., Hernández-C, J.I., Peck, O.C., et al. (1994). Temperature-metabolism relations in the cotton-top tamarin (*Saguinus oedipus*) model for ulcerative colitis. *J. Med. Primatol.* 23(1), 16-22. doi: 10.1111/j.1600-0684.1994.tb00090.x.
- Tattersall, G.J. (2012). "Diurnal changes in metabolic rate in pygmy marmosets: Implications for sleep, torpor, and basal metabolism in primates," in *Living in a Seasonal World*. Springer), 471-480.
- Toussaint, D.C., and McKechnie, A.E. (2012). Interspecific variation in thermoregulation among three sympatric bats inhabiting a hot, semi-arid environment. *J. Comp. Physiol. B.* 182(8), 1129-1140. doi: 10.1007/s00360-012-0683-6.
- van Oldenborgh, G.J., Drijfhout, S., van Ulden, A., Haarsma, R., Sterl, A., Severijns, C., et al. (2009). Western Europe is warming much faster than expected. *Clim. Past* 5(1), 1-12.
- Warren, R.D., and Crompton, R.H. (1998). Diet, body size and the energy costs of locomotion in saltatory primates. *Folia Primatol.* 69(Suppl. 1), 86-100. doi: 10.1159/000052701.
- Withers, P.C. (1992). *Comparative animal physiology*. Orlando, FL: Saunder College Publishing.
- Withers, P.C. (2001). Design, calibration and calculation for flow-through respirometry systems. *Aus. J. Zool.* 49(4), 445-461. doi: 10.1071/ZO00057.
- Withers, P.C., Cooper, C.E., Maloney, S.K., Bozinovic, F., and Cruz-Neto, A.P. (2016). *Ecological and environmental physiology of mammals*. Oxford University Press.
- Wright, P.C., Izard, M.K., and Simons, E.L. (1986). Reproductive cycles in *Tarsius bancanus*. *Am. J. Primatol.* 11(3), 207-215. doi: 10.1002/ajp.1350110302.
- Yoder, A.D. (2003). "The phylogenetic position of genus *Tarsius*: Whose side are you on?," in *Tarsiers: past, present, and future*, eds. P.C. Wright, E.L. Simons & S. Gursky. (New Brunswick: Rutgers University Press), 161-175.

Chapter Three - Using thermoregulatory profiles to assess climate change vulnerability in an arboreal tropical bat: heterothermy may be a pre-adaptive advantage

Shaun Welman^{1*}, Andrew A. Tuen² and Barry G. Lovegrove¹

¹ School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

² Institute of Biodiversity and Environmental Conservation, Universiti Malaysia, Sarawak, Malaysia

*Corresponding author: welmans@ukzn.ac.za

Note: The manuscript style has been formatted in American English

Abstract

Many tropical endotherms are already confronted by ambient temperatures (T_a s) close to their body temperature (T_b) and risk severe hyperthermia due to global warming. Tropical fruit bats play a vital role in the ecosystem and their absence could have dire consequences for ecosystem health. Many fruit bats have exposed roosting habits that increase their vulnerability to heat stress. Here, we sought to investigate the thermoregulatory capacity of wild caught lesser dog-faced fruit bats (*Cynopterus brachyotis*) from the Island of Borneo. These bats are generalist frugivores that contribute in seed dispersal and plant pollination, weigh 32g and roost in foliage. Physiologically, these bats employ adaptive heterothermy and they are also reported to have high T_b s relative to other bats, which should make them less susceptible to hyperthermia. We determined the effect of T_a (21-36°C) on metabolism, T_b and evaporative cooling. We also measured the T_a and relative humidity (RH) at capture sites. The bats displayed a seemingly narrow thermoneutral zone of $30 \pm 1^\circ\text{C}$, a basal metabolic rate of $5.60 \pm 0.26 \text{ W.kg}^{-1}$ ($1.01 \pm 0.05 \text{ ml O}_2\text{.g}^{-1}\text{.hr}^{-1}$) and a normothermic T_b of $32.5 \pm 0.3^\circ\text{C}$; all much lower than expected. Evaporative cooling was only effective at $T_a \leq 31^\circ\text{C}$, above which heat storage became apparent. Bats typically entered torpor at $T_a < 25^\circ\text{C}$ and thermoconformed (i.e. allowed their T_b to fluctuate with T_a) at $T_a > 32^\circ\text{C}$. The microclimate at capture sites remained cool ($T_a = 24\text{-}25^\circ\text{C}$) and humid (RH >90%). Our study supports the argument that tropical endotherms are susceptible to hyperthermia due to their low T_b . Further, we discuss the potential advantage of adaptive heterothermy in coping with short-term heatwaves. However, the future of these bats, and likely other low T_b species, depends primarily on their thermally buffered habitats and decisive conservation action is required to protect thermal refugia.

Keywords: hyperthermia, body temperature, torpor, global warming, tropics, fruit bats

Introduction

The tropics are thought to host the highest diversity of species on the planet (Rohde 1992, den Tex & Leonard 2013, Jablonski et al. 2013, Jansson et al. 2013), yet, we have a limited physiological understanding of tropical species (Lovegrove 2000, Dillon et al. 2010). There is now a sense of urgency to fill the gap in our knowledge as recent studies argue that i) the rate of climate niche evolution required to keep up with the predicted rate of climate change is four orders of magnitude greater than any rate observed in the past (Quintero & Wiens 2013), ii) the rate of global warming predicted by the IPCC may have been underestimated (Stroeve et al. 2007, van Oldenborgh et al. 2009, Natchimuthu et al. 2016), and iii) tropical species are most vulnerable to global warming (Huey et al. 2012, Lovegrove et al. 2014). Heat tolerance poses a rigid physiological limit to what animals may endure and is a useful tool to gauge species adaptability to global warming (Sherwood & Huber 2010). Many tropical and semi-tropical mammals have comparatively low and thermolabile body temperatures ($T_b < 36^\circ\text{C}$; Racey & Stephenson 1996, Lovegrove & Génin 2008, Stawski & Geiser 2012, Lovegrove et al. 2014). Consequently, they must endure relatively small temperature differentials between their T_b and ambient temperature (T_a). The smaller thermal gradient (i.e. low $\Delta T = T_b - T_a$) compromises their ability to passively dissipate excess body heat or maintain T_b within species-specific ranges because heat dissipation via conduction and radiation diminishes as ΔT approaches zero (Schmidt-Nielsen 1997, Sherwood & Huber 2010). By contrast, their reliance upon evaporative cooling, a physiologically active form of heat dissipation, increases as T_a increases (Tattersall et al. 2012).

Once $T_a \geq T_b$ evaporative cooling becomes the only means of dissipating heat from the body (McKechnie & Wolf 2004, Cory Toussaint & McKechnie 2012, Withers et al. 2012). Although evaporative water loss (EWL) may increase with T_a , the effectiveness of evaporative cooling is ultimately dependent on the water vapour pressure deficit between the animal and its surroundings (Schmidt-Nielsen 1997, Willmer et al. 2005). For any heat dissipation to occur there needs to be at least a $1\text{--}2^\circ\text{C}$ differential between the animal's skin and the wet-bulb temperature (Sherwood & Huber 2010). Thus, whereas mammals inhabiting higher latitudes usually encounter large water vapour pressure deficits that favour evaporative cooling (Walsberg 2000), the high relative humidity (RH) conditions in the tropics could potentially retard evaporative cooling and lead to fatal levels of heat storage (Mora et al. 2017). The rate at which animals store heat is size dependent. Due to a high surface-area-to-volume ratio the rate of heat exchange is greater in small mammals ($<1\text{ kg}$). As a result, small mammals have low thermal inertia and store heat faster (Schmidt-Nielsen 1997, Tattersall et al. 2012). Based on the above, Lovegrove et al. (2014) argued that arboreal tropical small mammals have the highest risk of suffering heat stress due to global warming. Arborealism is a high-risk factor as

several arboreal mammals lead exposed lifestyles and are thus susceptible to environmental perturbations (Bondarenco et al. 2014, Lovegrove et al. 2014).

Many tropical bats are small and roost in trees or other exposed sites (Kunz 1982, Kunz & Lumsden 2003, Santana et al. 2011), but are perceived to be less vulnerable to heat stress because they have the potential to migrate. Indeed, there is evidence of climate related range shifts by bats (Welbergen et al. 2008). Be that as it may, due to their nocturnal nature, bats are likely to endure day time conditions rather than relocate (but see Bondarenco et al. 2014). As such, the vulnerability of tree-roosting species to pathological hyperthermia should not be disregarded (Kunz 1982, Welbergen et al. 2008); case in point, the mass die-offs that have been observed in flying-foxes (*Pteropus* spp.) during heatwaves (Welbergen et al. 2008, Sherwin et al. 2013). Due to climate change, these extreme-weather events are likely to increase in frequency and severity, even within the tropics (Rohini et al. 2016, Russo et al. 2016). It is therefore concerning that many tropical bats have $T_b \leq 34^\circ\text{C}$ (see McNab 1969, McNab 1989, Soriano et al. 2002) and that tree-roosting is common among fruit-bats (family: Pteropodidae) (Kunz & Lumsden 2003, Santana et al. 2011). From an economic perspective, the monetary value of all ecosystem services rendered by bats is estimated to be upwards of \$250 billion U.S., with several \$100 million U.S. stemming from revenue linked to pollination and seed dispersal services of tropical fruit-bats alone (Fujita & Tuttle 1991, Kunz et al. 2011, Kasso & Balakrishnan 2013). From an ecological perspective, nectivorous and frugivorous bats play a vital role in maintaining and restoring plant communities by facilitating plant gene flow and aiding in seedling recruitment (Mello et al. 2011, Lewanzik & Voigt 2014, Preciado-Benítez et al. 2015, Abedi-Lartey et al. 2016). Given the significance of these bats, evaluating their chances of survival, as well as the ecological consequences of a population collapse or a migratory exodus should be a priority.

Bat-fruit networks are more robust than bird-fruit networks because they have a large overlap between interacting species (Fleming et al. 2009, Mello et al. 2011). However, as argued above, many fruit bats are hypothesised to be susceptible to hyperthermia and it is not yet clear how they will respond to global warming or climate change at large (Sherwin et al. 2013, O'Shea et al. 2016). Generalist species are expected to be less affected by climate change (Gilman et al. 2010, Hof et al. 2012, Gough et al. 2015, Afonso Silva et al. 2017) and are more likely to survive and persist within their current habitats. Thus, one potential outcome could be that generalist bats, although they are not the preferred plant companion, would serve as a safeguard in maintaining ecosystem integrity. Here, we focus on the lesser dog-faced fruit bat (*Cynopterus brachyotis*), a species that is likely to fulfil the role of a safeguard. *C. brachyotis* is a foliage-roosting generalist frugivore that is widely distributed throughout Asia (Liat 1970, Abdullah 2003). These bats are important seed dispersers that also participate in

pollination (Liat 1970, Boon & Corlett 1989, Tan et al. 1998). Thus, within their specific bat-fruit network, *C. brachyotis* could potentially substitute for specialists at either role. Relative to other bats, *C. brachyotis* has a high T_b ($T_b = 36.5^\circ\text{C}$; McNab 1989) and should maintain a favorable ΔT for longer than their lower T_b counterparts. In addition, these bats are heterothermic (McNab 1989) and torpor would enable them to conserve energy and water, and make them more resilient to climate change (Geiser & Turbill 2009, Geiser et al. 2011, Geiser & Brigham 2012). Considering the potential significance of *C. brachyotis* as a safeguard of the ecosystem (i.e. through their dual role in pollination and seed dispersal), we wanted to validate the T_b and metabolic rate thermoregulatory data reported by McNab (1989). In addition, we wanted to improve the existing understanding of thermoregulation of these bats by including the corresponding rate of evaporative heat loss (EHL) to provide better context for any changes in T_b or metabolism; which was previously absent. Specifically, we wanted to assess how core T_b , resting metabolic rate (RMR) and EHL of freshly-caught wild bats responded to changes in T_{as} of $21\text{--}36^\circ\text{C}$. Our aim was to use the interplay between these physiological variables to determine the onset of heat stress in the study population. Here, we assumed that heat stress occurred when metabolic heat production exceeded maximal evaporative heat loss, leading to increases in core T_b . This set of criteria was used to accommodate situations where animals tolerate moderate heat storage to avoid using body water to cool themselves. To afford the study animals the best opportunity to offload heat, especially at low ΔT , we conducted our experiments in a low humidity atmosphere. Based on the earlier argument that there needs to be at least a $1\text{--}2^\circ\text{C}$ differential between the animal's skin and the wet-bulb temperature, the fact that *C. brachyotis* has a normothermic T_b of 36.5°C , as well as the choice to expose bats to a low humidity atmosphere, we hypothesised that the onset of heat stress would occur at $T_a > 34^\circ\text{C}$. Furthermore, to gain insight about the bats natural setting and provide context for the laboratory data, we also documented T_a and RH at capture sites.

Materials and methods

Study site and animal capture

The study included 27 adult *C. brachyotis* (16 males, 11 non-pregnant females) with a mean body mass ($M_b \pm \text{SE}$) of $31.9 \pm 0.9\text{g}$ at first capture. Bats were caught in mist nets during sampling periods between August - October 2014 and March - August 2015 at four sites within the Sarawak Province, Borneo, Malaysia. Sixteen bats were caught at Mount Singai, five on the western slope (site 1, coordinates: $1^\circ 30' 20'' \text{N}$; $110^\circ 09' 22'' \text{E}$, $M_b = 28.2 \pm 0.9\text{g}$) and eleven on the eastern slope (site 2, coordinates: $1^\circ 30' 17'' \text{N}$; $110^\circ 10' 35'' \text{E}$, $M_b = 29.7 \pm 1\text{g}$). Six bats were caught at the Universiti Malaysia Sarawak (UNIMAS) campus (site 3, coordinates:

1°30'17" N; 110°10'35" E, $M_b = 34.8 \pm 1.9\text{g}$) and the remaining five bats were caught at Sama Jaya Nature Reserve (site 4, coordinates: 1°30'17" N; 110°10'35" E, $M_b = 36.8 \pm 2.1\text{g}$). Nets were periodically rotated between sites throughout trapping and were placed across streams, near fruiting trees and along clearings between potential roosting areas. All nets were opened shortly before dusk at ca. 18:00 and checked at regular intervals throughout the night. We used pre-calibrated HOBO temperature and relative humidity data loggers (Onset Computer Corporation, USA; resolution: $T_a = 0.02^\circ\text{C}$, $\text{RH} = 0.05\%$) to record the T_a and RH between 18 February 2015 and 15 June 2015 at capture sites 2 and 3. The loggers were installed at a height of ca. 1.5m above ground and set to record at 10min intervals.

Captured individuals were sexed, and thereafter weighed using a Pesola spring scale (Model: 20060, micro-line metric spring scale, 60g; Resolution: 0.5g). Morphometric measurements were also collected for use in a long-term monitoring program. To assess their pregnancy status, females were inspected for nipple swelling or vaginal perforation, and an abdominal palpation was conducted. Pregnant or lactating females were excluded from the study. After inspection, a temperature-sensitive passive integrated transponder (PIT) tag (Biomark HDX12, Boise, Idaho, USA; resolution: 0.1°C) was injected directly into each bats' intraperitoneal cavity to facilitate T_b measurements and serve as a unique identification marker. They were then hydrated and kept in cloth bags without access to food for six hours prior to respirometry measurements to ensure a post-absorptive status. Upon completion of respirometry measurements, bats were housed in a wire-mesh cage and provided with a mixture of locally available fruits (apple, banana and/or pineapple) and water. All animals spent a maximum of 36 hours in captivity and there were no fatalities as a consequence of the PIT tag procedure. Furthermore, even though no analgesic drugs were used to minimize handling time, no licking of the wound or other behaviour that would suggest irritation was observed afterwards.

At low T_{aS} , we used the bats post experimental behaviour to confirm that they were in torpor and not hypothermic. Hypothermic bats would remain lethargic for long periods due to the longer time required to rewarm. By contrast, bats in torpor would be able to arouse quickly, their T_b would increase rapidly and they would return to their normal responsive behavior. No hypothermic responses were observed.

Gas exchange measurements and experimental protocol

We used a flow-through respirometry system with an incurrent flow measurement design (Lighton 2008) to measure the rate of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and evaporative water loss (EWL) of bats exposed to T_{aS} between 21-36°C. During respirometry measurements, bats were housed individually in sealed 1l

respirometers constructed from glass jars. The roof of each respirometer was modified to allow the bats to hang in a natural position. A 1 cm layer of vegetable oil was poured into the base of each respirometer to prevent evaporation from urine and faeces affecting the readings. High vacuum grease (Dow Corning) was applied to all joints to ensure an air-tight seal and respirometers were tested for leaks before every measurement. The O₂ concentration within the respirometers were kept >20% by flowing dry CO₂-free air past the animal at constant flow rates between 150-400 ml.min⁻¹. Incurrent air was dried and scrubbed of CO₂ by drawing atmospheric air through a PC-4 Condensing Dryer (Sable Systems, Las Vegas, USA) and then columns of silica gel, indicating soda lime and indicating Drierite. Once dried, the airstream was split and one stream was blown through the respirometer past the animal, while the other stream was used as a reference airstream. Separate pump and flow meter units (SS-4 sub-sampler, Sable Systems) were used for each airstream. We used a RM-8 Flow Multiplexer (Sable Systems) to sequentially select between the reference stream and the excurrent air from the respirometer, which was sub-sampled and flowed through a series of gas analysers. A RH-300 water vapour analyser (Sable Systems) unit was used to measure the water vapor content of the subsampled air and a field gas analysis system (Foxbox-C, Sable Systems) measured the fractional concentrations of CO₂ and O₂. Water vapour and CO₂ were removed from the air once they had been measured and dry CO₂-free air flowed through the O₂ analyser. The digital outputs of the instruments were recorded on a personal laptop at 1-sec intervals using the Sable System's data acquisition software, Expedata (v 1.7.22), Baseline and lag corrections were performed in Expedata to synchronize the respective gas-exchange traces before any calculations were made.

We used the equations in Withers (2001) to calculate $\dot{V}O_2$, $\dot{V}CO_2$ and EWL from steady-state traces corresponding to the most level continuous 10min section of the $\dot{V}O_2$ trace. The associated M_b was calculated from a regression of the animals' M_b at the start and end of respirometry measurements. We calculated the respiratory exchange ratio as $\dot{V}CO_2/\dot{V}O_2$, and converted RMR from gas exchange measurements to a measure of energy (Watts) using the thermal equivalence data in Table 4.2 of Withers (1992). We also converted EWL to a measure of evaporative heat loss (Watts) using the standard conversion factor of 2.26 J. mg H₂O⁻¹. Thereafter, we calculated the amount of metabolic heat dissipated through evaporation as the ratio between EHL and the metabolic heat produced (MHP) through resting metabolism (EHL/MHP).

All measurements were conducted during the rest phase (06:00 – 18:00) and bats were exposed to a single T_a for 4-6 hrs at $T_a < 34^\circ\text{C}$. Following the death of a bat at $T_a = 35^\circ\text{C}$, the duration of exposure was reduced to 3 hrs at $T_a \geq 34^\circ\text{C}$. We are confident that bats were

heat stressed at $T_a \geq 34^\circ\text{C}$ within 3 hrs because they began to pant, lick and urinate on themselves if exposed for much longer; all of which are characteristic of animals in heat stress. Data from the dead bat were not used in calculations. Similarly, we excluded four potentially erroneous measurements of EWL (and thus EHL/MHP) from calculations. The T_a within respirometers were recorded using iButtons (DS1922L, Thermochron, Dallas, TX, USA; resolution: 0.0625°C) that were stuck to the underside of the lids and side walls. A Biomark HPR plus reader and antennae was used to read the PIT tags and record T_b at 30 sec intervals throughout the experiment. All iButtons and PIT tags were calibrated against a mercury thermometer, traceable to NIST standards, to the nearest 0.1°C . The CO_2 and water vapour analysers were calibrated monthly and the O_2 analyser was spanned before every measurement. The CO_2 analyser was calibrated using commercially available compressed gas and the water vapour analyser was calibrated using a bubbler flask and waterbath to generate humid air of a known dew point temperature.

Data analyses

We adopted the statistical approach and techniques used by Levesque et al. (2014). Briefly, we performed a piecewise regression analysis in R 3.0.2 (R Core Team 2017) using the package 'segmented' (Muggeo 2008) to identify any changes in the slope (i.e. points of inflection) along the respective thermoregulatory profiles of T_a versus RMR, T_b , EWL and EHL/MHP in non-torpid bats. Each segment of data identified through the analysis was treated as independent and we used simple linear regression analyses to determine the relationship between the physiological variable and T_a . We used mass-specific data to account for the effect of M_b on metabolism when conducting regression analyses. However, we also determined the relative importance of T_a , M_b , sex, and capture site on each physiology variable using mixed-effect models in the R package 'nlme' (Pinheiro et al. 2016). To account for repeated measurements we included individual ID as a random factor in all models. We determined the model of best-fit by comparing Akaike Information Criterion values corrected for small sample size [AICc and AICcWt; Burnham and Anderson (2003)] using the R package "AICcmodavg" (Mazerolle 2015). Model assumptions were verified using qq-plots and histograms of the residuals. All values are reported as mean \pm standard error unless stated otherwise.

All experimental protocols and permission to capture the bats were approved by the Sarawak Forestry Department [permit number: NCCD.907.4.4(9)-223, NCCD.907.4.4(13)-277]. All experimental and animal care procedures were also reported to and approved by the University of Kwazulu-Natal Animal Ethics Committee (116/13/Animal).

Results

RMR displayed a point of inflection at $T_a = 30.2 \pm 3.0^\circ\text{C}$ (\pm 95% confidence interval; CI) (Fig. 3-1a) and had a positive relationship T_a above the inflection ($F_{1,35} = 26.17$, $p < 0.01$, $r^2 = 0.44$; Table 3-1), but a negative relationship with T_a below the inflection ($F_{1,7} = 16.98$, $p < 0.01$, $r^2 = 0.74$; Table 3-1). The model of best-fit for RMR above the inflection included T_a as the only fixed factor (Akaike weight = 0.51; Table 3-2) whereas the model of best-fit below the inflection included all fixed effect factors except for sex (Akaike weight = 1; Table 3-2). To determine the thermoneutral zone (TNZ) we used an analysis of variance (ANOVA) to test if there was a difference between the minimum non-torpid RMR observed at $T_a = 29^\circ\text{C}$ and those observed at $T_a = 30\text{--}32^\circ\text{C}$. The ANOVA determined that there was a difference in RMR ($F_{1,16} = 16.29$, $p < 0.01$) and using a Tukey's HSD post hoc test we found that RMR at $T_a = 29^\circ\text{C}$ differed from those at $T_a = 32^\circ\text{C}$ ($p = 0.03$), but not those at $T_a = 30\text{--}31^\circ\text{C}$ ($p = 0.93\text{--}0.06$). Thus, we determined TNZ to be $29\text{--}31^\circ\text{C}$ and used the data therein to calculate a basal metabolic (BMR) of $5.60 \pm 0.26 \text{ W.kg}^{-1}$ ($1.01 \pm 0.05 \text{ ml O}_2\text{.g}^{-1}\text{.hr}^{-1}$). Because we did not have data at $T_a = 28^\circ\text{C}$, we attempted to resolve the lower limit of TNZ by determining the T_a at which BMR intersected the line describing the relationship between T_a and RMR at $T_a < 28^\circ\text{C}$. The intersection occurred at $T_a = 29.3^\circ\text{C}$ and supports our initial estimate of TNZ.

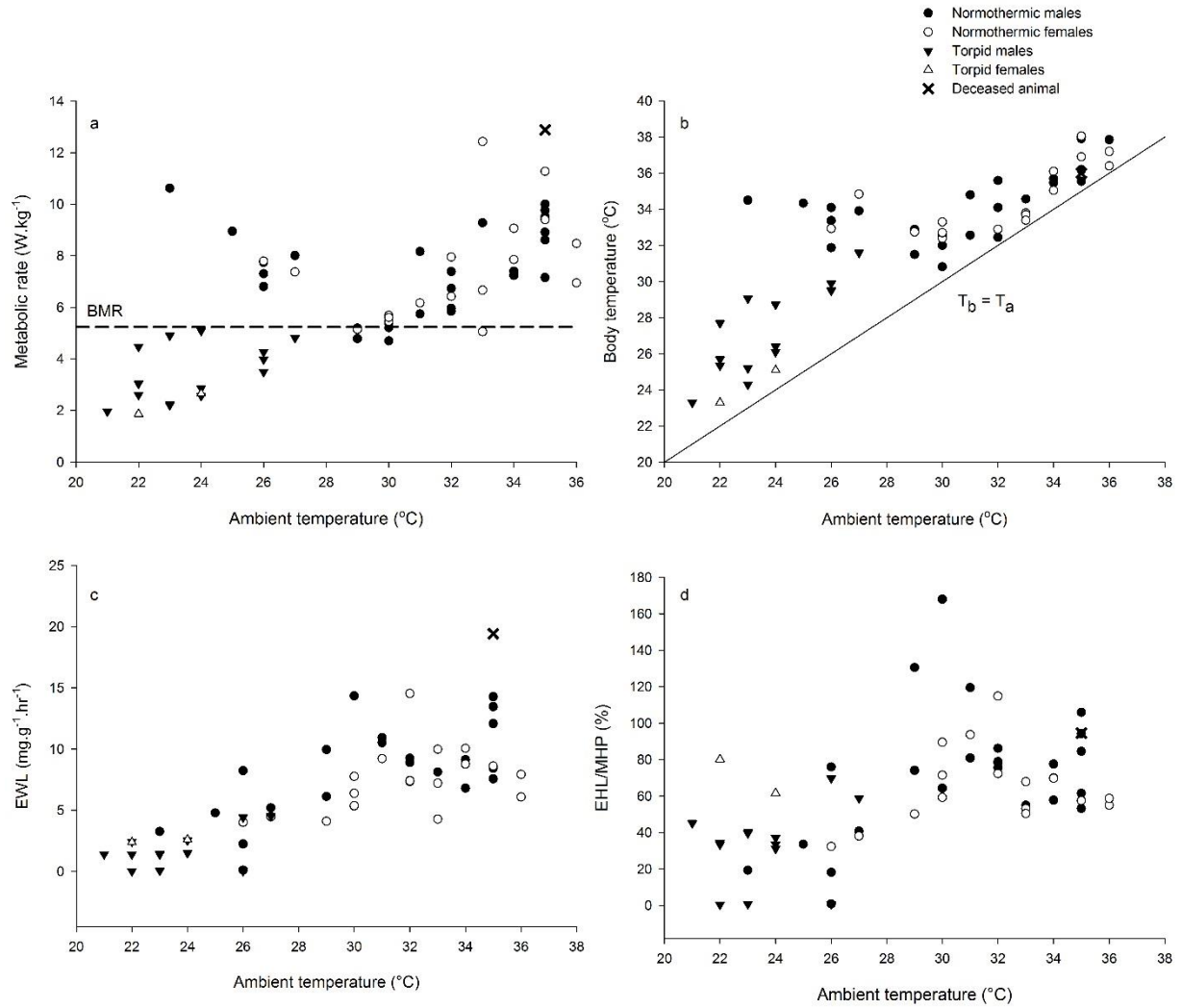


Figure 3-1: The thermoregulatory profile of *Cynopterus brachyotis*. a) Effect of ambient temperature (T_a) on resting metabolic rate. The dashed line indicates basal metabolic rate (BMR). b) Effect of T_a on core body temperature (T_b). The diagonal line indicates where body temperature equals ambient temperature ($T_b = T_a$). c) Effect of ambient temperature on the rate of evaporative water loss (EWL). d) Effect of T_a on the amount of metabolic heat dissipated through evaporative cooling i.e. the ratio of evaporative heat loss (EHL) to metabolic heat produced (MHP).

Table 3-1: The relationship between ambient temperature (T_a) and mass-specific resting metabolic rate (RMR), body temperature (T_b), the rate of evaporative water loss (EWL) and the amount of metabolic heat dissipated through evaporative cooling (EHL/MHP) in non-torpid *Cynopterus brachyotis*. ** $p < 0.01$.

Parameter	T_a (°C)	Measures included	Regression equation
Mass-specific RMR	< 28	Normothermic only	$RMR = 28.873 - 0.808 \times T_a^{**}$
	≥ 30	All	$RMR = 0.671 \times T_a - 14.496^{**}$
T_b	23–36	Normothermic only	$T_b = 0.3679 \times T_a + 22.919^{**}$
EWL	23–36	Normothermic only	$EWL = 0.6197 \times T_a - 11.2893^{**}$
EHL/MHP	≤ 30	Normothermic only	$EHL/MHP = 12.67323 \times T_a - 290.493^{**}$

Both T_b ($F_{1,43} = 31.100$, $p < 0.01$, $r^2 = 0.42$; Fig. 3-1b, Table 3-1) and EWL ($F_{1,40} = 19.13$, $p < 0.01$, $r^2 = 0.32$; Fig. 3-1c, Table 3-1) had a positive relationship with T_a and there were no inflection points in either variable. The model of best-fit for T_b included T_a and capture site as the only fixed effect factors (Akaike weight = 0.40; Table 3-2), whereas the model of best-fit for EWL included T_a and M_b as the only fixed effect factors (Akaike weight = 0.63; Table 3-2). Due to the thermolabile nature of the bats, we determined a normothermic T_b of $32.5 \pm 0.3^\circ\text{C}$ from bats measured within the TNZ.

EHL/MHP displayed a single point of inflection at $T_a = 30.1 \pm 1.8^\circ\text{C}$ ($\pm 95\%$ CI) and had a positive relationship with T_a until the point of inflection ($F_{1,15} = 10.57613$, $p < 0.01$, $r^2 = 0.43$; Table 3-1), but there was no relationship with T_a thereafter. The model of best-fit for EHL/MHP included T_a as the only fixed effect factor (Akaike weight = 0.55; Table 3-2).

T_a at capture sites displayed a unimodal right skewed distribution (Fig. 3-2). The overall range in T_a at capture site 2 was 21.4°C to 30.0°C and the modal T_a was 24.1°C . The overall range in T_a at capture site 3 was 21.6°C to 31.2°C and the modal T_a was 24.3°C . Only ca. 1hr 25min and ca. 6hrs 22min of the 118 days of recording at capture site 2 and 3 respectively, was spent at $T_a \geq 30^\circ\text{C}$. By contrast, the RH at both sites remained high throughout the recorded period, with RH $\approx 100\%$ observed for 90% of the time at capture site 2 and for 68% of the time at capture site 3. The lowest RH recorded at capture site 2 was 77.8% and the lowest RH at capture site 3 was 58.6 %.

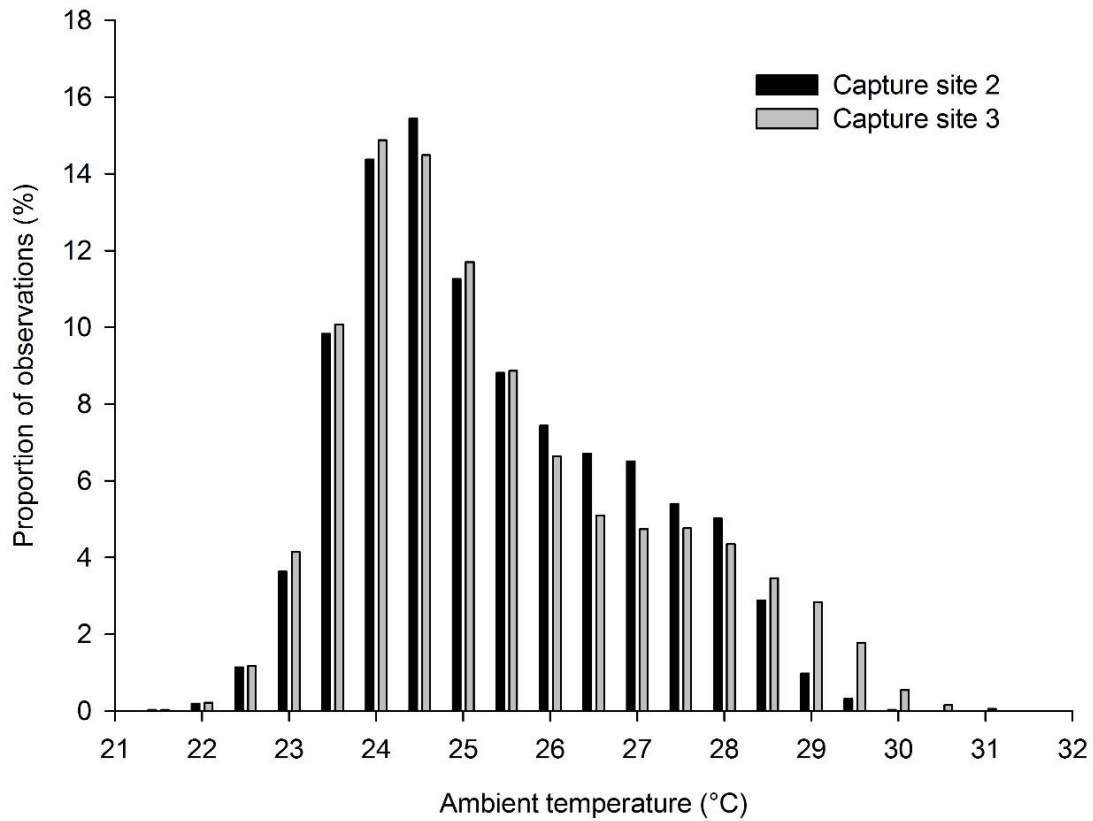


Figure 3-2: Ambient temperature (T_a) recorded at capture sites between 18 February 2015 and 15 June 2015. Capture site 2: eastern slope of Mount Singai; capture site 3: Universiti Malaysia Sarawak campus. Data are presented as a percentage of the total number of recordings.

Table 3-2: Best-fitting linear mixed-effect models evaluating the influence of ambient temperature (T_a), body mass (M_b), sex and capture site on resting metabolic rate (RMR), body temperature (T_b), the rate of evaporative water loss (EWL) and the amount of metabolic heat dissipated through evaporative cooling (EHL/MHP) in non-torpid *Cynopterus brachyotis*. k = the number of parameters for the model. AICc = Akaike's information criterion values corrected for small sample size. Only models with Akaike weights >0.01 are presented.

Fixed factors	Random factor	k	AICc	Akaike weights
RMR ($T_a > 30^\circ\text{C}$)				
T_a	~1 ID	4	-138.29	0.51
$T_a + M_b$	~1 ID	5	-136.52	0.21
$T_a + \text{Sex}$	~1 ID	5	-136.41	0.20
$T_a + M_b + \text{Sex}$	~1 ID	6	-134.42	0.07
$T_a + \text{Capture site}$	~1 ID	6	-129.78	0.01
RMR ($T_a < 28^\circ\text{C}$)				
$T_a + M_b + \text{Capture site}$	~1 ID	8	-189.34	1
T_b				
$T_a + \text{Capture site}$	~1 ID	7	169.02	0.40
$T_a + M_b + \text{Capture site}$	~1 ID	8	170.27	0.21
T_a	~1 ID	4	171.51	0.12
$T_a + \text{Sex} + \text{Capture site}$	~1 ID	8	171.95	0.09
$T_a + M_b$	~1 ID	5	172.68	0.06
$T_a + M_b + \text{Sex} + \text{Capture site}$	~1 ID	9	173.39	0.05
$T_a + \text{Sex}$	~1 ID	5	173.69	0.04
$T_a + M_b + \text{Sex}$	~1 ID	6	174.12	0.03
EWL				
$T_a + M_b$	~1 ID	5	209.37	0.63
$T_a + M_b + \text{Sex}$	~1 ID	6	211.41	0.23
$T_a + \text{Capture site}$	~1 ID	7	214.71	0.04
$T_a + M_b + \text{Capture site}$	~1 ID	8	214.96	0.04
$T_a + M_b + \text{Sex} + \text{Capture site}$	~1 ID	9	215.65	0.03
$T_a + \text{Sex} + \text{Capture site}$	~1 ID	8	215.69	0.03
EHL/MHP ($\leq 30^\circ\text{C}$)				
T_a	~1 ID	4	167.17	0.55
$T_a + \text{Sex}$	~1 ID	5	168.36	0.30
$T_a + M_b$	~1 ID	5	170.11	0.13
$T_a + M_b + \text{Sex}$	~1 ID	6	173.67	0.02

Discussion

Our results did not support the hypothesis that the onset of heat stress would occur at $T_a > 34^\circ\text{C}$. Instead, the onset of heat stress occurred at ca. 32°C . Furthermore, our T_b data contradict those of McNab (1989). We found that normothermic bats typically maintained a T_b of 32.5°C , which is ca. 3.1°C lower than the T_b reported previously. Whereas McNab (1989) measured rectal temperature as a proxy measure for core T_b , we measured core T_b directly. Thus, the discrepancy could be due to methodological differences. However, because T_b and BMR are correlated (Lovegrove 2003, 2005, Clarke et al. 2010), our observation that bats maintained a BMR that was ca. 79% of the BMR reported previously, provides corroborating support for the low T_b reported here. Furthermore, our results support the argument that tropical species, given their generally low T_b , are more vulnerable to global warming thanks to their susceptibility to heat stress. Here, because of their low T_b , the ability of *C. brachyotis* to defend normothermia was compromised at seemingly moderate T_a s and heat storage occurred at $T_a \geq 31^\circ\text{C}$. As expected, the bats displayed a thermoregulatory increase in evaporative cooling as T_a increased and could dissipate ca. 98% of their resting MHP through evaporation at $\Delta T = 1^\circ\text{C}$. However, metabolic processes are temperature sensitive (Withers 1992, Tattersall et al. 2012) and it appears that once $T_a \approx T_b$, the increase in MHP due to Arrhenius effects overcame their capacity for evaporative cooling; despite any increase in EWL. Hence, the results of our study suggests that *C. brachyotis*, at least the individuals studied here, are physiologically more susceptible to hyperthermia than expected based on previous accounts of their T_b .

The inability of the bats to defend normothermia beyond $\Delta T = 1^\circ\text{C}$, within a dry atmosphere, is concerning. Due to the high humidity in the field, the results suggest that heat storage is likely to develop even before $T_a = 31^\circ\text{C}$. Fortunately, capture sites remained cool and forest T_a seldom exceeded 30°C . *C. brachyotis* are primarily foliage-roosting tent-making bats (Campbell et al. 2006) and recent work suggests that tent shelters may not be as effective at buffering high T_a as they are at buffering low T_a (Rodríguez-Herrera et al. 2016). Tents that are constructed in open areas are hotter and are more variable because they are exposed to solar radiation (Rodríguez-Herrera et al. 2016). Thankfully, the cooler conditions closer to the ground afford the bats the option to exploit roosts that are less exposed and more stable in temperature. In addition to lowering their risk of hyperthermia, exploiting thermally stable roosts would reduce their daily energy expenditure (Rodríguez-Herrera et al. 2016) and bolster the benefits gained through torpor.

As mentioned earlier, torpor is a highly effective mechanism of energy and water conservation and significantly improves the survivability of mammals (Geiser & Turbill 2009,

Geiser & Stawski 2011, Stawski et al. 2014, Bondarenko et al. 2016). It lowers the risk of extinction in bats because it reduces their risk of predation and increases their reproductive success by enabling them to manipulate pregnancy and delay fetal development during periods of unfavorable environmental conditions (Geiser & Brigham 2012, Stawski et al. 2014). In addition, recent work suggests that torpor may also afford bats greater resiliency to heat stress. Bondarenko et al. (2014) reported that one little broad-nosed bat (*Scotorepens greyii*) and two inland freetail bats (*Mormopterus* species 3), both of which are heterotherms, managed to survive the extreme T_a during a desert heat wave. The authors noted that the bats thermoconformed at high T_a and argued that, by doing so, they could reduce their water loss and prevent dehydration. Here, we observed that *C. brachyotis* had a similar ability to thermoconform at high T_a and it is possible that they did so to prevent dehydration, but we add that torpor likely also reduced the bats heat stress by lowering the contribution of MHP to the overall heat budget. Evidence of torpor use at high T_a is growing and has been reported in eastern pygmy-possums (*Cercartetus nanus*; Song et al. 1997), spiny mice (*Acomys russatus*, Grimpo et al. 2013) and grey short-tailed opossums (*Monodelphis domestica*; Busse et al. 2014). Given the benefits of torpor, it seems that heterothermic species have a pre-adaptive advantage over their homeothermic counterparts when dealing with climate change.

We acknowledge that phenotypic plasticity may have contributed to some of the physiological differences observed between bats in our study and those of McNab (1989). In this regard, there are attempts to determine the influence of phenotypic plasticity on species responses to climate change (Canale & Henry 2010, Chevin et al. 2010, Boyles et al. 2011). However, given that physiological tolerances limit adaptability (Sherwood & Huber 2010) and our limited understanding of torpor at high T_a , we argue that *C. brachyotis*, at least the bats in our study area, are physiologically susceptible to heat stress. Bats are already threatened by climate change (Sherwin et al. 2013) and plant-animal interactions are severely threatened by a range of anthropogenic related activities such as land use changes, species eradication and phenological mismatches due to climate change (Garrett et al. 2006, Abrol 2012, Sherwin et al. 2013). For example, land use changes have been extensive throughout the tropics. Within the South East Asian region, much of the natural vegetation has been converted to industrial plantations, particularly for use as oil palm plantations (Miettinen et al. 2016). The land use change on the Island of Borneo has been particularly aggressive (Reynolds et al. 2011) and there are concerns about the effect that it may have on atmospheric composition (Pyle et al. 2011, Warwick et al. 2013). It is estimated that 76% of Borneo was covered by old-growth forests during 1973, but that 34% of this land had been cleared by 2015 (Gaveau et al. 2016). The island is shared by three sovereign states namely Indonesia, Malaysia and Brunei, and the majority of the deforestation occurred in Indonesian Borneo, followed by Malaysian Borneo

and comparatively little in Brunei (Gaveau et al. 2016). In addition to reducing the available area for animals to live in, deforestation increases habitat fragmentation; which significantly alters the climate within forests and could make them less hospitable (Turner & Corlett 1996, Laurance 2004, Tuff et al. 2016). Therefore, it is crucial, especially in situations where the land is shared, to preemptively earmark suitable areas for conservation and protect thermal refugia (Struebig et al. 2010, Morelli et al. 2016). Even though bat-fruit networks are robust, the consequences of a population collapse or a migratory mass exodus of bats to distant most suitable areas, could bear significant consequences on the ecosystem.

Acknowledgements

We thank Luisa Duya Setia, Cecilia Emang Ajeng, Rahah Binti Mohamad Yakup, Mohamad Hasri Al-Hafiz Bin Haba, Danielle Levesque, Mary Buloh Balang, Yap Pui Kwan, Matthew Jenang and Tunmise Otitoju for their help in the field. We are also grateful to the Bidayuh communities of the Barieng and Tanjong villages in Singai for access to their forest and use of their facilities. The research was supported by the National Research Foundation (South Africa), GreenMatter fellowship (South Africa) and their partners, as well as the Ministry of Higher education of Malaysia (FRGS/1/2013/ST03/UNIMAS/01/2).

Literature cited

- Abdullah MT (2003) Biogeography and variation of *Cynopterus brachyotis* in Southeast Asia. PhD thesis, University of Queensland
- Abedi-Lartey M, Dechmann DKN, Wikelski M, Scharf AK, Fahr J (2016) Long-distance seed dispersal by straw-coloured fruit bats varies by season and landscape. *Glob Ecol Conserv* 7:12-24
- Abrol DP (2012) Climate change and pollinators. In: Abrol DP (ed) *Pollination biology: biodiversity conservation and agricultural production*. Springer, Dordrecht, p 479-508
- Afonso Silva AC, Bragg JG, Potter S, Fernandes C, Coelho MM, Moritz C (2017) Tropical specialist vs. climate generalist: diversification and demographic history of sister species of *Carlia* skinks from northwestern Australia. *Mol Ecol* 26:4045-4058
- Bondarenko A, Körtner G, Geiser F (2014) Hot bats: extreme thermal tolerance in a desert heat wave. *Naturwissenschaften* 101:679-685
- Bondarenko A, Körtner G, Geiser F (2016) How to keep cool in a hot desert: torpor in two species of free-ranging bats in summer. *Temperature* 3:476-483
- Boon PP, Corlett RT (1989) Seed dispersal by the lesser short-nosed fruit bat (*Cynopterus brachyotis*, Pteropodidae, Megachiroptera). *Malayan Nat J* 42:251-256

- Boyles JG, Seebacher F, Smit B, McKechnie AE (2011) Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr Comp Biol* 51:676-690
- Burnham KP, Anderson DR (2003) Model selection and multimodel inference: a practical information-theoretic approach, Vol 2. Springer, New York
- Busse S, Lutter D, Heldmaier G, Jastroch M, Meyer C (2014) Torpor at high ambient temperature in a neotropical didelphid, the grey short-tailed opossum (*Monodelphis domestica*). *Naturwissenschaften* 101:1003-1006
- Campbell P, Reid NM, Zubaid A, Adnan AM, Kunz TH (2006) Comparative roosting ecology of *Cynopterus* (Chiroptera: Pteropodidae) fruit bats in peninsular Malaysia. *Biotropica* 38:725-734
- Canale CI, Henry PY (2010) Adaptive phenotypic plasticity and resilience of vertebrates to increasing climatic unpredictability. *Clim Res* 43:135-147
- Chevin LM, Lande R, Mace GM (2010) Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLOS biology* 8:e1000357
- Clarke A, Rothery P, Isaac NJB (2010) Scaling of basal metabolic rate with body mass and temperature in mammals. *J Anim Ecol* 79:610-619
- Cory Toussaint D, McKechnie AE (2012) Interspecific variation in thermoregulation among three sympatric bats inhabiting a hot, semi-arid environment. *J Comp Physiol B*, 182:1129-1140
- den Tex RJ, Leonard JA (2013) A molecular phylogeny of Asian barbets: speciation and extinction in the tropics. *Mol Phylogenet Evol* 68:1-13
- Dillon ME, Wang G, Huey RB (2010) Global metabolic impacts of recent climate warming. *Nature* 467:704-706
- Fleming TH, Geiselman C, Kress WJ (2009) The evolution of bat pollination: a phylogenetic perspective. *Ann Bot* 104:1017-1043
- Fujita MS, Tuttle MD (1991) Flying Foxes (Chiroptera: Pteropodidae): threatened animals of key ecological and economic importance. *Cons Biol* 5:455-463
- Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE (2006) Climate change effects on plant disease: genomes to ecosystems. *Annual review of phytopathology* 44:489-509
- Gaveau DLA, Sheil D, Husnayaen, Salim MA and others (2016) Rapid conversions and avoided deforestation: examining four decades of industrial plantation expansion in Borneo. *Scientific reports* 6:32017

- Geiser F, Turbill C (2009) Hibernation and daily torpor minimize mammalian extinctions. *Naturwissenschaften* 96:1235-1240
- Geiser F, Stawski C (2011) Hibernation and torpor in tropical and subtropical bats in relation to energetics, extinctions, and the evolution of endothermy. *Integr Comp Biol* 51:337-348
- Geiser F, Stawski C, Bondarenko A, Pavey C (2011) Torpor and activity in a free-ranging tropical bat: implications for the distribution and conservation of mammals? *Naturwissenschaften* 98:447-452
- Geiser F, Brigham RM (2012) The other functions of torpor. In: Ruf T, Bieber C, Arnold W, Millesi E (eds) *Living in a seasonal world: thermoregulatory and metabolic adaptations*. Springer Heidelberg, Berlin, p 109-121
- Gilman SE, Urban MC, Tewksbury J, Gilchrist GW, Holt RD (2010) A framework for community interactions under climate change. *Trends Ecol Evol* 25:325-331
- Gough LA, Sverdrup-Thygeson A, Milberg P, Pilskog HE, Jansson N, Jonsell M, Birkemoe T (2015) Specialists in ancient trees are more affected by climate than generalists. *Ecol Evol* 5:5632-5641
- Grimpo K, Legler K, Heldmaier G, Exner C (2013) That's hot: golden spiny mice display torpor even at high ambient temperatures. *J Comp Physiol B* 183:567-581
- Hof AR, Jansson R, Nilsson C (2012) Future climate change will favour non-specialist mammals in the (sub)arctics. *PLOS ONE* 7:e52574
- Huey RB, Kearney MR, Krockenberger A, Holtum JA, Jess M, Williams SE (2012) Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Phil Trans R Soc B* 367:1665-1679
- Jablonski D, Belanger CL, Berke SK, Huang S and others (2013) Out of the tropics, but how? Fossils, bridge species, and thermal ranges in the dynamics of the marine latitudinal diversity gradient. *Proc Natl Acad Sci USA* 110:10487-10494
- Jansson R, Rodríguez-Castañeda G, Harding LE (2013) What can multiple phylogenies say about the latitudinal diversity gradient? a new look at the tropical conservatism, out of the tropics, and diversification rate hypotheses. *Evolution* 67:1741-1755
- Kasso M, Balakrishnan M (2013) Ecological and economic importance of bats (Order Chiroptera). *ISRN Biodivers* 2013:187415
- Kunz TH (1982) Roosting ecology of bats. In: Kunz TH (ed) *The ecology of bats*. Plenum Press, New York, p 1-55

- Kunz TH, Lumsden LF (2003) Ecology of cavity and foliage roosting bats. In: Kunz TH, Fenton MB (eds) Bat ecology. University of Chicago Press, Chicago, IL, p 3-89
- Kunz TH, Braun de Torrez E, Bauer D, Lobo T, Fleming TH (2011) Ecosystem services provided by bats. *Ann N Y Acad Sci* 1223:1-38
- Laurance WF (2004) Forest-climate interactions in fragmented tropical landscapes. *Phil Trans R Soc B* 359:345-352
- Levesque DL, Lobban KD, Lovegrove BG (2014) Effects of reproductive status and high ambient temperatures on the body temperature of a free-ranging basoendotherm. *J Comp Physiol B* 184:1041-1053
- Lewanzik D, Voigt CC (2014) Artificial light puts ecosystem services of frugivorous bats at risk. *J Appl Ecol* 51:388-394
- Liat LB (1970) Food habits and breeding cycle of the Malaysian fruit-eating bat, *Cynopterus brachyotis*. *J Mammal* 51:174-177
- Lighton JRB (2008) Measuring metabolic rates: a manual for scientists, Vol. Oxford University Press, New York
- Lovegrove BG (2000) The zoogeography of mammalian basal metabolic rate. *Am Nat* 156:201-219
- Lovegrove BG (2003) The influence of climate on the basal metabolic rate of small mammals: a slow-fast metabolic continuum. *J Comp Physiol B* 173:87-112
- Lovegrove BG (2005) Seasonal thermoregulatory responses in mammals. *J Comp Physiol B* 175:231-247
- Lovegrove BG, Génin F (2008) Torpor and hibernation in a basal placental mammal, the Lesser Hedgehog Tenrec *Echinops telfairi*. *J Comp Physiol B* 178:691-698
- Lovegrove BG, Canale C, Levesque DL, Fluch G, Rehakova-Petru M, Ruf T (2014) Are tropical small mammals physiologically vulnerable to arrhenius effects and climate change? *Physiol Biochem Zool* 87:30-45
- Mazerolle MJ (2015) AICcmodavg: model selection and multimodel inference based on (Q)AIC(c). R package version 2.0-3
- McKechie AE, Wolf BO (2004) Partitioning of evaporative water loss in white-winged doves: plasticity in response to short-term thermal acclimation. *J Exp Biol* 207:203-210
- McNab BK (1969) The economics of temperature regulation in neotropical bats. *Comp Biochem Physiol* 31:227-268

- McNab BK (1989) Temperature regulation and rate of metabolism in 3 Bornean bats. *J Mammal* 70:153-161
- Mello MAR, Marquitti FMD, Guimarães PR, Kalko EKV, Jordano P, de Aguiar MAM (2011) The missing part of seed dispersal networks: Structure and robustness of bat-fruit interactions. *PLOS ONE* 6:e17395
- Miettinen J, Shi C, Liew SC (2016) Land cover distribution in the peatlands of Peninsular Malaysia, Sumatra and Borneo in 2015 with changes since 1990. *Glob Ecol Conserv* 6:67-78
- Mora C, Dousset B, Caldwell IR, Powell FE and others (2017) Global risk of deadly heat. *Nat Clim Change* 7:501-506
- Morelli TL, Daly C, Dobrowski SZ, Dulen DM and others (2016) Managing climate change refugia for climate adaptation. *PLOS ONE* 11:e0159909
- Muggeo VMR (2008) Segmented: an R package to fit regression models with broken-line relationships. *R news* 8:20-25
- Natchimuthu S, Sundgren I, Gålfalk M, Klemetsson L, Crill P, Danielsson Å, Bastviken D (2016) Spatio-temporal variability of lake CH₄ fluxes and its influence on annual whole lake emission estimates. *Limnol Oceanogr* 61:S13-S26
- O'Shea TJ, Cryan PM, Hayman DTS, Plowright RK, Streicker DG (2016) Multiple mortality events in bats: a global review. *Mamm Rev* 46:175-190
- Pinheiro J, Bates D, DebRoy S, Deepayan S, Team RC (2016) *nlme: linear and nonlinear mixed effects models*. R package version 3.1-128
- Preciado-Benítez O, Gómez y Gómez B, Navarrete-Gutiérrez DA, Horváth A (2015) The use of commercial fruits as attraction agents may increase the seed dispersal by bats to degraded areas in Southern Mexico. *Trop Conserv Sci* 8:301-317
- Pyle JA, Warwick NJ, Harris NRP, Abas MR and others (2011) The impact of local surface changes in Borneo on atmospheric composition at wider spatial scales: coastal processes, land-use change and air quality. *Phil Trans R Soc B* 366:3210-3224
- Quintero I, Wiens JJ (2013) Rates of projected climate change dramatically exceed past rates of climatic niche evolution among vertebrate species. *Ecol Lett* 16:1095-1103
- R Core Team (2017) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria

- Racey PA, Stephenson PJ (1996) Reproductive and energetic differentiation of the Tenrecidae of Madagascar. In: R. LW (ed) Biogéographie de Madagascar, Orstom, Paris, p 307-319
- Reynolds G, Payne J, Sinun W, Mosigil G, Walsh RPD (2011) Changes in forest land use and management in Sabah, Malaysian Borneo, 1990–2010, with a focus on the Danum Valley region. *Phil Trans R Soc B* 366:3168-3176
- Rodríguez-Herrera B, Viquez-R L, Cordero-Schmidt E, Sandoval JM, Rodríguez-Durán A (2016) Energetics of tent roosting in bats: the case of *Ectophylla alba* and *Uroderma bilobatum* (Chiroptera: Phyllostomidae). *J Mammal* 97:246-252
- Rohde K (1992) Latitudinal Gradients in Species Diversity: The Search for the Primary Cause. *Oikos* 65:514-527
- Rohini P, Rajeevan M, Srivastava AK (2016) On the Variability and Increasing Trends of Heat Waves over India. *Sci Rep* 6:26153
- Russo S, Marchese AF, Sillmann J, Immé G (2016) When will unusual heat waves become normal in a warming Africa? *Environ Res Lett* 11:054016
- Santana SE, Dial TO, Eiting TP, Alfaro ME (2011) Roosting ecology and the evolution of pelage markings in bats. *PLOS ONE* 6:e25845
- Schmidt-Nielsen K (1997) Animal physiology: adaptation and environment, Vol 5. Cambridge University Press, Cambridge
- Sherwin HA, Montgomery WI, Lundy MG (2013) The impact and implications of climate change for bats. *Mamm Rev* 43:171-182
- Sherwood SC, Huber M (2010) An adaptability limit to climate change due to heat stress. *Proc Natl Acad Sci USA* 107:9552-9555
- Song X, Körtner G, Geiser F (1997) Thermal relations of metabolic rate reduction in a hibernating marsupial. *Am J Physiol Regul Integr Comp Physiol* 273:R2097-R2104
- Soriano PJ, Ruiz A, Arends A (2002) Physiological Responses to Ambient Temperature Manipulation by Three Species of Bats from Andean Cloud Forests. *J Mammal* 83:445-457
- Stawski C, Geiser F (2012) Will temperature effects or phenotypic plasticity determine the thermal response of a heterothermic tropical bat to climate change? *PLOS ONE* 7:e40278
- Stawski C, Willis CKR, Geiser F (2014) The importance of temporal heterothermy in bats. *J Zool* 292:86-100

- Stroeve J, Holland MM, Meier W, Scambos T, Serreze M (2007) Arctic sea ice decline: Faster than forecast. *Geophys Res Lett* 34:L09501
- Struebig M, Christy L, Pio D, Meijaard E (2010) Bats of Borneo: diversity, distributions and representation in protected areas. *Biodivers Conserv* 19:449-469
- Tan KH, Zubaid A, Kunz TH (1998) Food habits of *Cynopterus brachyotis* (Muller) (Chiroptera: Pteropodidae) in Peninsular Malaysia. *J Trop Ecol* 14:299-307
- Tattersall GJ, Sinclair BJ, Withers PC, Fields PA, Seebacher F, Cooper CE, Maloney SK (2012) Coping with thermal challenges: Physiological adaptations to environmental temperatures. *Compr Physiol* 2:2151-2202
- Tuff KT, Tuff T, Davies KF (2016) A framework for integrating thermal biology into fragmentation research. *Ecol Lett* 19:361-374
- Turner I, Corlett RT (1996) The conservation value of small, isolated fragments of lowland tropical rain forest. *Trends Ecol Evol* 11:330-333
- van Oldenborgh GJ, Drijfhout S, van Ulden A, Haarsma R and others (2009) Western Europe is warming much faster than expected. *Clim Past* 5:1-12
- Walsberg GE (2000) Small Mammals in hot deserts: Some generalizations revisited. *BioScience* 50:109-120
- Warwick NJ, Archibald AT, Ashworth K, Dorsey J and others (2013) A global model study of the impact of land-use change in Borneo on atmospheric composition. *Atmos Chem Phys* 13:9183-9194
- Welbergen JA, Klose SM, Markus N, Eby P (2008) Climate change and the effects of temperature extremes on Australian flying-foxes. *Proc R Soc B* 275:419-425
- Willmer P, Stone G, Johnston I (2005) *Environmental physiology of animals*, Vol. Blackwell publishing, Oxford
- Withers PC (1992) *Comparative animal physiology*. Orlando, FL: Saunder College Publishing.
- Withers PC (2001) Design, calibration and calculation for flow-through respirometry systems. *Aus J Zool* 49:445-461
- Withers PC, Cooper CE, Nespolo RF (2012) Evaporative water loss, relative water economy and evaporative partitioning of a heterothermic marsupial, the monito del monte (*Dromiciops gliroides*). *J Exp Biol* 215:2806-2813

Chapter Four - Torpor at high temperatures? Thermoregulatory comparison between heterothermic and homeothermic bats.

Shaun Welman^{1*} and Barry G. Lovegrove¹

¹ School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

*Corresponding author: welmans@ukzn.ac.za

Note: The manuscript style has been formatted in American English

Abstract

Support for the idea that animals use metabolic down-regulation at high temperature, hereafter “hyperthermic torpor”, is growing. Given the influence that it could have on an endotherm’s response to global warming, we sought to find corroborating physiological evidence to support claims of hyperthermic torpor. We chose bats as our model because they show plasticity in their use of torpor, many display characteristics of putative hyperthermic torpor such as thermoneutral zones that extend far beyond their normothermic body temperature (T_b), and there is a large dataset on thermoregulatory variables available for bats. Contemporary models may be unable to allow a direct determination of hyperthermic torpor, so an indirect approach was adopted. In this study, we define adaptive heterothermy as the ability to enter torpor or hibernation, and use this definition to delineate bat species into two groups. We term bats that can use torpor or hibernation as heterotherms, whereas bats that have yet to be seen to enter torpor or hibernation are termed homeotherms. We would have preferred to use thermoregulatory responses coupled with a measure of operative T_a s instead of T_a , but such data are largely unavailable. Nonetheless, we compared the lower- (T_{lc}) and upper (T_{uc}) limits of thermoneutrality between heterothermic and homeothermic bats, as well as their T_b and rate of evaporative water loss (EWL) at these limits. Heterotherms ($24.2 \pm 4.3g$, $n = 18$) were smaller than homeotherms ($61.2 \pm 16.8g$, $n = 11$) but, once phylogeny was considered, mass had no effect. The two groups of bats had similar T_{lc} s ($28.9 \pm 0.6^\circ C$ vs $29.0 \pm 1.0^\circ C$) and T_{uc} s ($35.1 \pm 0.9^\circ C$ vs $35.0 \pm 0.7^\circ C$), but heterotherms maintained lower T_b s (T_b at T_{lc} : $33.6 \pm 0.5^\circ C$ vs $36.9 \pm 0.3^\circ C$; T_b at T_{uc} : $36.7 \pm 0.6^\circ C$ vs $39.1 \pm 0.4^\circ C$). Crucially, heterotherms displayed no additional investment in evaporative cooling even though they should have, to deal with expected heat storage at $T_{uc} > T_b$ (EWL at T_{uc} : $2.3 \pm 1.2 \text{ mg H}_2\text{O} \cdot g^{-1} \cdot hr^{-1}$ vs $7.5 \pm 3.6 \text{ mg H}_2\text{O} \cdot g^{-1} \cdot hr^{-1}$; $n = 5$ per group). Apart from evaporative cooling, the only other mechanism that could have retarded heat storage at T_{uc} , was to alter their heat balance

by using hyperthermic torpor. Given that our results are consistent with the use of hyperthermic torpor, at least in bats, we also discuss the potential biochemical mechanisms that could facilitate such a metabolic reduction.

Keywords: Hyperthermia, Torpor, Thermoregulation, Bats, Global warming

Introduction

Much of our understanding surrounding the physiological effects of temperature on endotherms has been driven by the need to understand their response to cold (Angilletta Jr et al., 2010; Lovegrove, 2017). Consequently, our understanding of thermoregulatory responses to high ambient temperatures (T_{as}), that is, at T_{as} where animals face hyperthermia, is considerably more limited; especially for mammals. There is a sense of urgency to fill this gap given the rapid rate of global warming. It has been argued that most mammals will not be able to adapt physiologically, morphologically or behaviorally to conditions predicted for the future (Rahmstorf et al., 2007; Quintero and Wiens, 2013). As global warming continues, many species will experience increasing heat stress. Certainly, most extant mammals have never encountered the extreme heat conditions that are predicted to occur within the next century.

The rate of heat exchange between the environment and an animal is dependent on the animal's size (Schmidt-Nielsen, 1997; Tattersall et al., 2012). Whereas mammals of intermediate (1-10 kg) and large (>10 kg) body sizes benefit from high thermal inertia, small mammals (<1 kg) do not (Lovegrove, 2005). Given the thermodynamic effects due to body size and that larger mammals also have more total body water available to use for evaporative cooling, mammals of varying body sizes are likely to exhibit different physiological responses to global warming, which may ultimately affect their overall response (McCain and King, 2014). Small mammals, especially those with relatively low body temperatures ($T_b < 36^\circ\text{C}$, Lovegrove et al., 2014a), seem particularly susceptible to heat stress, thus for the purposes of our study we restrict our focus to them; but see studies by Fuller et al. (2010), Hetem et al. (2014) and Fuller et al. (2016) for discussions on the potential responses of large mammals to global warming.

To cope with environmental challenges, many small mammals rely on a facultative down-regulation of metabolism known as adaptive heterothermy (torpor or hibernation), or other behavioural mechanisms such as huddling to reduce their energy expenditure during episodes of energetic bottlenecks (Heldmaier, 1989; Lovegrove, 2005). However, even though we have a comprehensive understanding of the benefits of adaptive heterothermy at low T_{as} (Hallam and Mzilikazi, 2011; Geiser and Brigham, 2012; Dausmann, 2014; Stawski et al.,

2014), and have identified a growing list of species that are heterothermic (McKechnie and Mzilikazi, 2011; Lovegrove, 2012; Ruf and Geiser, 2015), the potential use of hypometabolism and the benefit(s) thereof are poorly understood at high T_a s. Does adaptive heterothermy provide an advantage for dealing with heat?

By entering a hypometabolic state at high T_a , hereafter, referred to as “hyperthermic torpor” to differentiate it from the typical hypometabolic state at low T_a , mammals could certainly benefit from a reduction in water loss if there was also an associated abandonment of the defense of normothermic T_b (Bondarenko et al., 2014). Here, normothermic T_b refers to the core temperature ± 1 SD measured in a postabsorptive individual at rest and exposed to thermoneutral zone T_a s (IUPS Thermal Commission, 2003). Case in point, by thermoconforming a little broad-nosed bat (*Scotorepens greyii*; $M_b = 7.2$ g) and two inland freetail bats (*Mormopterus* species 3 (Adams et al., 1988); $M_b = 7.8$ g) endured a 3-day long desert heatwave wherein T_a peaked at ca. 48°C (Bondarenko et al., 2014). Should this observation by Bondarenko et al. (2014) represent a legitimate metabolic down-regulation, it would bolster the argument that adaptive heterothermy significantly improves survivability (Nowack et al., 2015; Doty et al., 2016; Nowack et al., 2016) and thus reduces extinction risk (Geiser and Turbill, 2009). It would also lend credence to the argument that adaptive heterothermy allowed mammals to survive the environmental turmoil at the Cretaceous-Palaeogene boundary (Lovegrove et al., 2014b).

Given our limited understanding of hyperthermic torpor (Song et al., 1997; Grimpot et al., 2013; Bondarenko et al., 2014; Busse et al., 2014; O'Mara et al., 2017) and the potential role it may have regarding species response to climate change (reviewed by Nowack et al., 2017), we conducted a meta-analysis on mammalian thermoregulatory profiles to determine whether there was any corroborating physiological evidence to support the idea that heterotherms down-regulate metabolism at T_a s typically associated with hyperthermia, as indicated by heat storage. Our hypothesis hinges on differences that we predict should occur in the physiological response to hyperthermia between heterothermic and homeothermic species. The assumption that we make is that heterothermic species already have the metabolic machinery required for metabolic down-regulation, albeit at low T_a , and that this would enable them to down-regulate metabolism at high T_a as well, whereas homeothermic species do not have the metabolic machinery needed to down-regulate metabolism.

Modeling hyperthermic torpor

Torpor, at low T_a , is typically identified when metabolic rate decreases to less than the species basal metabolic rate (BMR) measured at thermoneutrality. Unfortunately, unlike during torpor at low T_a , a direct determination of metabolic suppression at high T_a is challenging. It requires that the proportion of metabolic rate increase related to increased efforts at evaporative cooling, such as an elevated heart rate and panting, is separated from passive metabolic rate increases related to Arrhenius effects (but see O'Mara et al., 2017). Furthermore, even if this physiological entanglement is overlooked, modeling tools such as the Arrhenius and Van't Hoff equations (Arrhenius, 1915; Lloyd and Taylor, 1994), and simplified derivations thereof, such as Q_{10} (Geiser, 1988):

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\frac{10}{T_{b2} - T_{b1}}} \quad \text{Equation 1}$$

where R_1 and R_2 represent an endotherm's metabolic rate measured at T_{b1} and T_{b2} respectively, or the simplified Newtonian equation used by McNab (1980):

$$MR = C(T_b - T_a) \quad \text{Equation 2}$$

where MR represents an endotherm's metabolic rate, C its thermal conductance and $T_b - T_a$ is the temperature differential between the endotherm and the ambient; are all unreliable comparative measures at high T_a . This is because endotherms actively regulate T_b , or the equations become unrealistic as T_a approaches T_b , i.e. as $\Delta(T_b - T_a) \rightarrow 0$, or further still because thermal conductance (C) is not truly constant. However, by investigating variations in T_b and the interplay between the physiological variables that affect heat storage, it may be possible to indirectly establish whether some degree of metabolic down-regulation at high T_a occurs.

Endotherms that attempt to defend normothermia in response to changes in T_a typically display a sinusoidal T_b pattern across a broad range of T_a s (Lovegrove et al., 1991a; Lovegrove et al., 1991b). Their slightly elevated T_b at the colder range of tolerable T_a s occurs because the rate of heat production temporally exceeds the rate of heat loss. However, further decreases in T_a leads to a decrease in T_b as the rate of heat loss overcomes the animals' capacity for endogenous heat production.

Modelling the physiological factors affecting body temperature

The rate at which animals store heat may be modelled by rearranging the simplified heat balance equation of Schmidt-Nielsen (1997):

$$T_S = H_{MR} \pm H_C \pm H_R \pm H_E \quad \text{Equation 3}$$

where T_S is heat storage, H_{MR} is heat production from metabolic processes (always positive), H_C is the net heat exchange from the combined effects of conductive and convective heat transfer, H_R is the net heat exchange from radiation, and H_E the net heat exchange from evaporation (typically negative).

Maintaining a species-specific normothermic T_b requires a variable, but somewhat predictable, energetic investment (H_{MR}) to regulate T_s such that T_b is constant; conveniently described by the Scholander-Irving (SI) model of thermoregulation (Scholander et al., 1950). The SI model characterizes a range of T_a s wherein adjustments in C are sufficient to maintain normothermia with no additional metabolic investment (Fig. 4-1a), known as the thermoneutral zone (TNZ). Defending normothermia at T_a s above or below TNZ requires metabolic investment. Furthermore, should an endotherm attain a minimum C at $T_a \leq T_{lc}$, then the SI model predicts a relationship between metabolism and $T_a \leq T_{lc}$ that is typically linear. Extrapolating the relationship to the x-axis i.e. rearranging equation 2 and setting $MR = 0$, $T_a = T_b$: which represents the animal's theoretical setpoint T_b . It is also noteworthy that C , in the context of the SI model, is an analogue of the net effect of the H_C , H_R and H_E components in the heat balance equation (Equation 3) (Schmidt-Nielsen, 1997; Tattersall et al., 2012).

The rate of dry thermal conductance (i.e. H_C and H_R) increases from the lower critical limit (T_{lc}) of TNZ and typically reaches its maximum rate at TNZ's upper critical limit (T_{uc}) (Tattersall et al., 2012). However, the capacity to passively dissipate excess body heat by dry thermal conductance diminishes as the thermal gradient between the animal and T_a is reduced (Schmidt-Nielsen, 1997; Tattersall et al., 2012). Once $T_a \approx T_b$, dry thermal conductance becomes ineffective at off-loading body heat and mammals rely entirely on evaporative cooling to retard heat storage (i.e. T_S) (Fig. 4-1b) (Sherwood and Huber, 2010; Cory Toussaint and McKechnie, 2012; Tattersall et al., 2012). Because body water is limited, mammals tend to allow their T_b to increase slightly at relatively moderate T_a s to maintain a favorable thermal gradient against which heat can be offloaded passively, thereby allowing them to reduce their evaporative water loss and conserve body water (Schmidt-Nielsen, 1997; Tattersall et al., 2012). Due to Arrhenius effects, an elevated T_b should equate to an increased metabolic rate, unless there is some form of metabolic down-regulation that off-sets the increase. It is this interplay of variables that forms the basis of our comparison.

lower dashed lines). The dark-blue and red shaded sections represent the range of metabolic rates required to defend normothermia. The light-blue section illustrates the potential influence of evaporative cooling on thermal conductance at high T_a s. The drop-lines are color coded to indicate the corresponding T_a at the lower (T_{lc}) and upper (T_{uc}) limits of the TNZ. The grey shaded section represents the range of metabolic rates and T_a s over which heterotherms typically employ torpor or hibernation, that is, the abandonment of normothermia. b) The effect of evaporative cooling on the T_b profile of an endotherm defending normothermia. The red line represents the likely T_b profile in the absence of evaporative cooling, whereas the black line represents the T_b profile associated with moderate evaporative cooling and the light-blue line represents the T_b profile with effective evaporative cooling. The horizontal dashed lines indicate the level of the normothermic (lower) and lethal (upper) T_b s respectively. The dark-blue drop-line indicates T_{lc} and the drop-lines indicating T_{uc} are color coded to the T_b profiles.

Modelling considerations

Based on the SI model, for any given value of C , species with higher basal metabolic rates (BMRs) should display broader TNZs relative to those with lower BMRs (Fig. 4-1a). One would also expect that T_{uc} should be higher in species with higher T_{bs} (Fig. 4-2), firstly because T_b is correlated to BMR even though the relationship may not be straight-forward (Lovegrove, 2003; 2005; Clarke and Pörtner, 2010; Clarke et al., 2010), and secondly because the higher thermal gradient would allow passive heat loss at higher T_{as} and delay the metabolic investment related to active cooling and Arrhenius effects due to increased heat storage (Schmidt-Nielsen, 1997; Tattersall et al., 2012; Fristoe et al., 2015). However, it may be possible that mammals can extend T_{uc} and reduce heat storage with increases in evaporative cooling that is not reflected by an increase in metabolism (Fig. 4-1a, b). For instance, the inflection in EWL marking the onset of evaporative cooling occurs well before TNZ in some species (Hosken and Withers, 1997; Marom et al., 2006) or at T_{lc} in others (Hosken and Withers, 1999; Cooper and Withers, 2012), yet metabolism remains the same throughout TNZ irrespective of whether EWL is low or moderate.

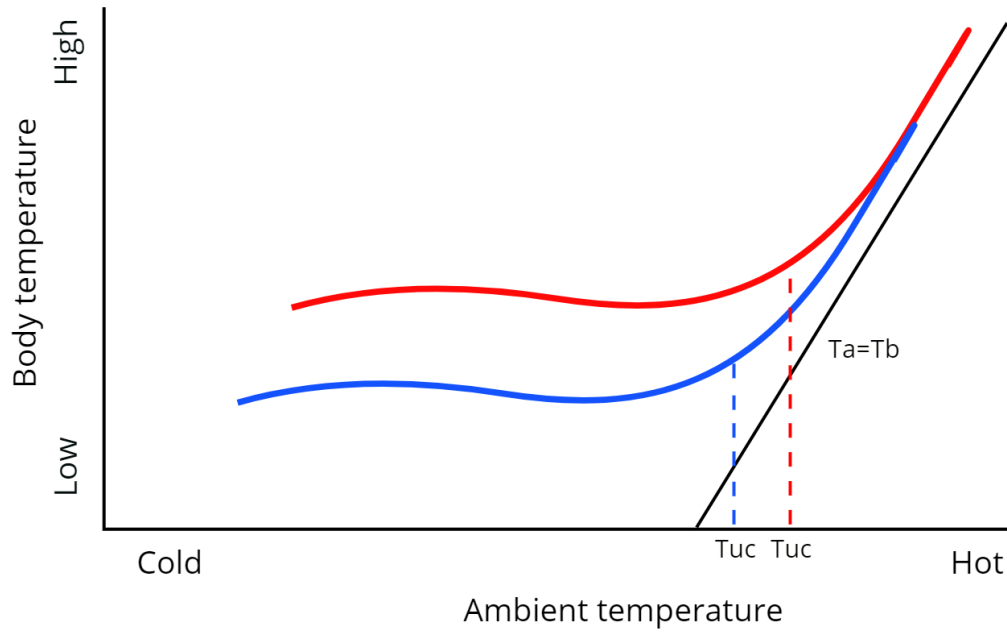


Figure 4-2: A schematic model of the theoretical difference in the upper critical limit (T_{uc}) of the thermoneutral zone between endotherms that have similar capacities for thermal conductance and evaporative cooling, but one has a low (blue line) normothermic body temperature (T_b) and the other has a high normothermic T_b (red line). The model illustrates that the thermal gradient which facilitates passive heat dissipation, i.e. $T_b - T_a$ becomes unfavorable for heat loss at a relatively lower ambient temperature, resulting in an earlier onset of heat storage with increasing T_a .

Hyperthermic Torpor Model predictions

By reducing metabolism at $T_a > T_{lc}$, heterothermic species could lower the endogenous component of the heat balance equation (i.e. H_{MR}), lower their heat loading, retard heat storage at moderate T_a or prevent dehydration at high T_a ; hence delay the onset of severe heat stress. However, a visible reduction in metabolic rate would likely be obscured due to the opposing influence of the Arrhenius effect forcing an increase in metabolism, while metabolic down-regulation serves to decrease metabolism. This paradox could potentially explain why heterothermic bats such as Angolan Free-tailed bats (*Mops condylurus*, $T_b = 35.2^\circ\text{C}$, mass = 23.2g; Maloney et al., 1999), sooty mustached bats (*Pteronotus quadridens*, $T_b = 31.2^\circ\text{C}$, mass = 4.8g; Rodríguez-Durán, 1995) and Antillean ghost-faced bats (*Mormoops blainvilli*, $T_b = 32.0^\circ\text{C}$, mass = 8.6g; Rodríguez-Durán, 1995) display extremely broad ($>10^\circ\text{C}$) TNZs given their small size, and seemingly have a $T_{uc} > 40^\circ\text{C}$. Similar though less extreme observations were also made for other heterothermic bats, namely Gould's wattled bats (*Chalinolobus gouldii*, $T_b = 35.0^\circ\text{C}$, mass = 17.5g; Hosken and Withers, 1997), lesser long-eared bats (*Nyctophilus geoffroyi*, $T_b = 35.9^\circ\text{C}$, mass = 8.0g; Hosken and Withers, 1999), European free-tailed bats (*Tadarida teniotis*, $T_b = 35.1^\circ\text{C}$, mass = 32.0g; Marom et al., 2006) and Hemprich's long-eared bats (*Otonycteris hemprichii*, $T_b = 35.6^\circ\text{C}$, mass = 25g; Marom et al., 2006) where T_{uc} exceeded normothermic T_b by at least 4°C .

Due to the challenges of direct determination of metabolic down-regulation at high T_a , we adopted an indirect approach and examined the interplay between T_b , evaporative water loss (EWL) and the critical limits of TNZ as proxy measures of putative hyperthermic torpor. We chose bats as our study model because 1) they display a remarkable ability to exploit adaptive heterothermy in response to a diverse range of environmental stressors (Geiser and Brigham, 2012; Stawski et al., 2014), 2) there are several studies that report thermoregulatory profiles wherein bats display characteristics of putative hyperthermic torpor (as discussed above), and 3) there is a large dataset on thermoregulatory variables available for comparison. Specifically, we tested whether heterothermic and homeothermic species of bats differed with respect to the mean temperature at which T_{lc} and T_{uc} occurred, whether they differed in terms of the mean T_b at these limits, and if so, whether one group displayed a larger increase in T_b between T_{lc} and T_{uc} , which would indicate more heat storage. We also tested whether the two groups of bats differed in the rate of EWL at both T_{lc} and T_{uc} to provide further context of any observed heat storage. We developed three *a priori* model predictions that are not necessarily mutually exclusive of each other, but all independently support our hypothesis that heterothermic species use hyperthermic torpor. The models also account for potential

variations between target variables that are unrelated to metabolism. For example, the two groups of bats could have similar thermal limits but different T_b s and thus different levels of heat storage that are explained by differences in evaporative cooling or other metabolic-independent factors (Fig. 4-3).

After accounting for potential body mass (M_b) and phylogenetic effects, we predicted that, if metabolic suppression did occur, then heterothermic species could display one of the following responses:

1. A higher T_{uc} with similar T_b s and rate of EWL (Fig. 4-3a); where hyperthermic torpor would be manifested as an extension of T_{uc} in heterotherms. In this scenario, Arrhenius effects would mask a visible reduction of metabolism, T_b and EWL at $T_{as} > T_{lc}$, but because of the reduction in H_{MR} the escalation of metabolism and T_b , as seen at $T_{as} > T_{uc}$, would occur at a relatively higher T_a in heterotherms. Thus, due the counteracting effects of hyperthermic torpor and Arrhenius effects, the $T_b - T_a$ gradient is sustained at higher T_{as} and less evaporative cooling is required to defend normothermic T_b .

2. A lower T_b at similar T_{uc} s and a similar rate of EWL (Fig. 4-3b); where hyperthermic torpor would manifest as a reduced heat shortage leading to lower T_b s without additional investment in evaporative cooling i.e. both groups have equal rates of EWL. In this scenario, the reduction in H_{MR} would alter the heat balance, but the inflection point of metabolism would be at similar T_{as} , and heterotherms would display lower T_b s not extended T_{uc} s. At T_{uc} , dry thermal conductance is maximized and apart from a reduction in H_{MR} , evaporative cooling would be the only other means of reducing T_b .

3. Similar T_b s at similar T_{uc} s and a lower rate of EWL (Fig. 4-3c); where hyperthermic torpor would manifest as a significant reduction in EWL. In this scenario, Arrhenius effects would mask the visible reduction of both metabolism and T_b , resulting in similar metabolic rate and T_b profiles, but the reduction in H_{MR} would alter the heat balance such that, unlike scenario two, heterotherms would require a significantly lower investment in evaporative cooling to maintain similar T_b s. The benefit would be that heterotherms should be able to conserve water at moderately hyperthermic T_{as} , and thus have a larger water reserve available for use at more severe T_{as} .

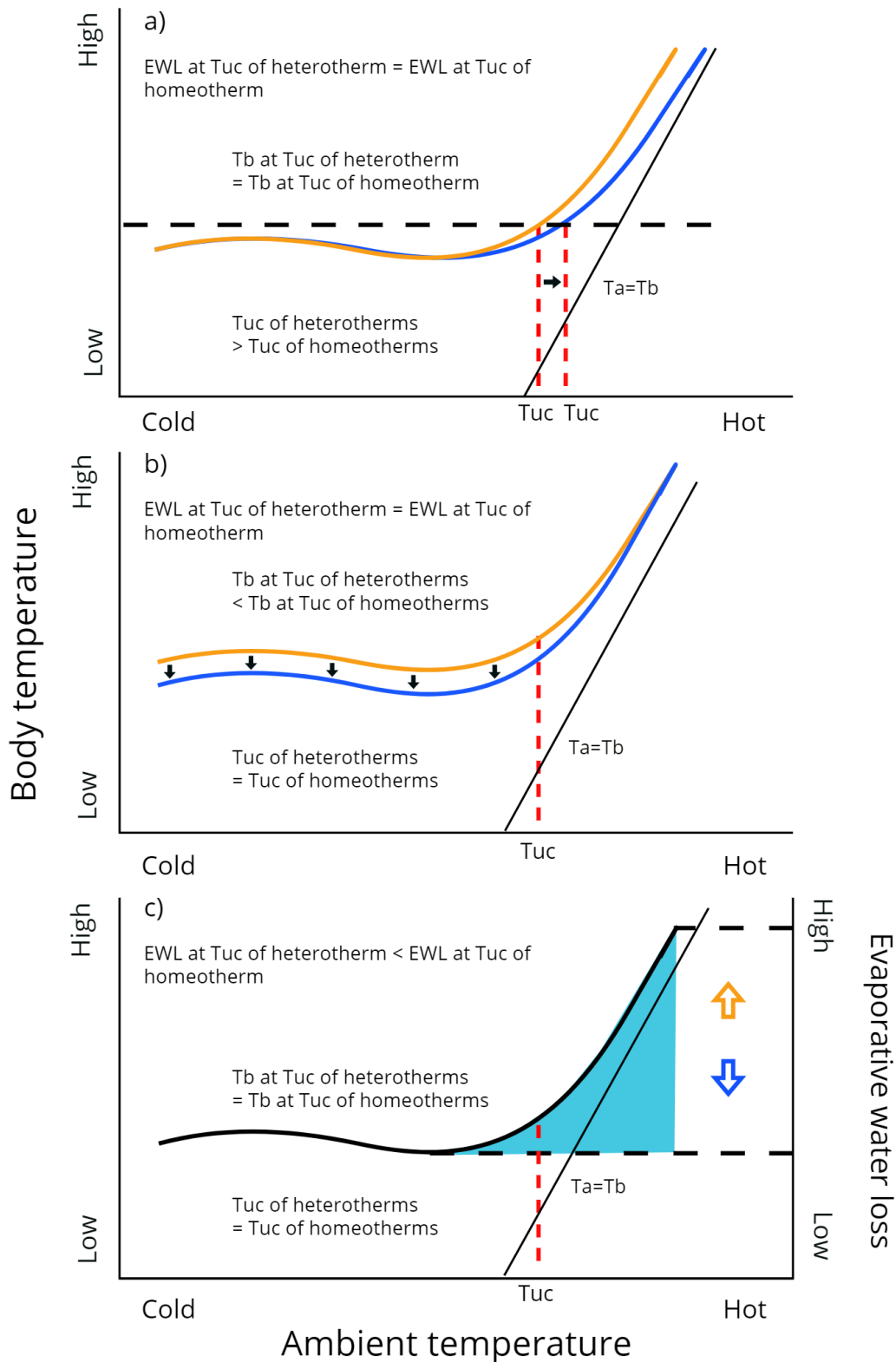


Figure 4-3: Theoretical models of the thermoregulatory adjustments in body temperature (T_b), upper critical limit of thermoneutrality (T_{uc}) and evaporative water loss (EWL) predicted by metabolic down-regulation in heterotherms at high ambient temperatures (T_{as}). The dark-blue

line represents the T_b profile of heterotherms, the brown line represents the T_b profile of homeotherms, the black line represents a common T_b profile between homeotherms and heterotherms a) The predicted upward shift in T_{uc} (black arrow) by heterotherms to a higher T_a due to metabolic down-regulation reducing the animal's internal heat component and facilitating similar T_b s (indicated by the dashed line) at higher T_a s without the influence of evaporative cooling. b) The predicted shift in the T_b profile (black arrows) of heterotherms due to metabolic down-regulation reducing the animal's internal heat component and facilitating lower T_b s at similar T_{uc} s without the influence of evaporative cooling. c) The predicted lower rate of EWL by heterotherms (dark-blue open arrow) relative to homeotherms (brown open arrow) at similar T_b s due to metabolic down-regulation reducing the animal's internal heat component and thus requiring a lower rate of evaporative cooling to maintain a given T_b . The light-blue shade indicates the range of potential rates of EWL.

Materials and methods

Dataset

We share the concerns of Wolf et al. (2017) who argued that systematic errors in datasets negatively influence the integrity of meta-analyses and weakened their credibility. Thus, we opted to generate a new dataset of physiological variables instead of adding to existing datasets such as Riek and Geiser (2013) or Khaliq et al. (2014). Indeed, some of the data compiled by both (and other) studies may not be accurate representations of the species' thermoregulatory capacity. For example, the widely reported $T_{uc} = 32.5^{\circ}\text{C}$ for Gould's long-eared bat (*Nyctophilus gouldi*) simply represents the highest experimental temperature to which the bats were exposed, and there are no supporting metabolic data. The original study shows that the bats' metabolism at the stated T_{uc} was no different to any other metabolic rate measurement made within the TNZ (see Geiser and Brigham, 2000). A similar anomaly is the $T_{uc} = 33^{\circ}\text{C}$ typically reported for the long-tongued nectar bat (*Macroglossus minimus*) which again represents the highest experimental temperature of that study and the single metabolic rate datum at 33°C does not differ in an obvious manner from the measurements at 32°C (see Bartels et al., 1998).

To generate our dataset, we first compiled a list of all relevant original sources cited in previous datasets and then performed additional literature searches to include recent studies. We only considered studies that presented concurrent measures of metabolic rate and T_b measured during the animals' rest phase. Thereafter, we refined our list of studies by excluding all studies where $n = 1$ or where the species metabolic rate profile included fewer than 10 data points in total. Of the remaining studies, where possible, we obtained raw data directly from authors, otherwise the data were mined from the original sources. During the data mining process, all metabolic rate, T_b and EWL profiles were digitized manually using PlotDigitizer 2.6.8 October 27, 2015 (Huwaldt and Stenhorst, 2015). For the sake of continuity and to minimize potential biases that could be introduced by multiple data collectors, each with their own discretion, the same investigator (SW) digitized all of the data. Unless stated otherwise we obtained M_b and corresponding normothermic T_b values from the same study.

When the original sources reported mean values instead of individual data points, we generated a random number set with the same mean value and margin of error at each T_a along the profile. When seasonal data were available, only the summer dataset was used. When gender specific data were available, the respective male and female measurements were combined to form a single species-specific profile for each variable of interest. When

species data were available from multiple studies, we based selection on the number of samples reported per T_a , as well as the range of T_a s measured. To standardize the metabolic rate data across studies, all values were converted to mass-specific oxygen consumption ($\text{mLO}_2\cdot\text{g}^{-1}\text{hr}^{-1}$). When necessary, if no respiratory exchange ratio (RER) value was reported, we assumed a value of 0.85 (Withers, 1992). To standardize the EWL data across studies, all values were converted to mass-specific values ($\text{mg H}_2\text{O}\cdot\text{g}^{-1}\text{hr}^{-1}$). When necessary, we used the standard heat of vaporization of water ($2.26 \text{ J}\cdot\text{mg H}_2\text{O}^{-1}\cdot\text{g}^{-1}$) as the conversion factor.

Calculating thermal limits

Our literature search yielded 70 candidate species and we visually inspected each thermoregulatory profile to exclude data obtained from animals that were obviously torpid during measurements. All statistical procedures were performed using R version 3.3.3. (R Core Team, 2017). We recalculated thermal limits for each species by reanalyzing the metabolic profiles using the R package “Segmented” (Muggeo, 2008) to apply a piecewise linear regression analysis and assumed that inflection points accurately represented the limits of TNZ. Where the piecewise analysis could not identify thermal limits, we performed a secondary analysis in which we calculated a mean metabolic rate and 95 % confidence intervals (CI calculated as $\text{mean} \pm 1.96 \times \text{standard error}$; Hackshaw, 2009) at each T_a . Starting from the lowest RMR (i.e. BMR), we compared the means at each T_a , in sequence, to determine the T_a at which metabolism deviated beyond the 95 % confidence range of BMR. We assumed that the highest and lowest T_a s whereat RMR was within the 95 % confidence range of BMR represented the respective limits of TNZ.

Based on the approach described above, we determined both T_{lc} and T_{uc} for 29 species, of which there were T_b data for all and EWL data for ten. To determine the corresponding T_b at each thermal limit, we conducted a linear regression analysis (piecewise or ordinary least squares [OLS]) on the T_b profiles of those 29 species and used their respective T_{lc} and T_{uc} values together with the regression statistics to calculate T_b (Table 4-1). We repeated this process to determine the rate of EWL at T_{lc} and T_{uc} , but fitted an exponential regression model to the data instead of a linear model (Baudinette et al., 2000) (Table 4-1).

Phylogenetic analysis

We used the time-calibrated species-level bat phylogeny of Shi and Rabosky (2015) for all phylogenetic analyses. The NEXUS file was obtained from the TreeBASE repository (www.treebase.org, reference number 17613) and the phylogeny containing 812 species of bats was pruned to include only the species used in our study using the R package “ape”

(Paradis et al., 2004). We then tested the variables in our dataset for evidence of phylogenetic signal using Blomberg et al.'s (Blomberg et al., 2003) K -statistic and P -values using the R package "picante" (Kembel et al., 2010), where K -values less than one indicates that closely related species resemble each other less than expected under a Brownian motion evolutionary model. K -values greater than one were taken to indicate that closely related species resemble each other more than expected. To visualize the phylogenetic relationship between the bats' and their T_b s at T_{lc} and T_{uc} relative to each other, we superimposed and plotted T_b onto a pruned phylogeny (Fig. 4-4) using the R package "phytools" (Revell, 2012).

The M_b of bats in our dataset ranged from the 4-g little forest bat (*Eptesicus vulturinus*; Willis et al., 2005) to the 404.3-g Moluccan naked-backed fruit bat (*Dobsonia moluccensis*; McNab and Bonaccorso, 2001) (Table 4-1). All M_b data were \log_{10} -transformed to normalize them before analyses. We determined the influence of M_b on all physiological variables by comparing the outputs of OLS regression analyses to the outputs of phylogenetic generalized least squares (PGLS) regression analyses conducted in the R package "caper". Based on the negligible influence of M_b on physiological variables after accounting for phylogeny (see Results section), we used a phylogenetic Analysis of Variance (phyloANOVA) to compare the two groups of bats and test for differences in T_b , T_{lc} , T_{uc} , T_b at T_{lc} , T_b at T_{uc} , as well as the difference in T_b between the thermal limits (ΔT_b). The comparative data are presented as means \pm standard error.

Following Riek and Geiser (2013), all calculations involving T_{lc} were expressed as $T_b - T_{lc}$ to deal with potential variations in BMR and thermal conductance (see equation 3). Curiously, for *Tadarida brasiliensis*, the normothermic $T_b = 29^\circ\text{C}$ reported by Soriano et al. (2002) was less than T_{lc} (Table 4-1). It is illogical for endotherms to maintain a normothermic $T_b < T_{lc}$ because increases in metabolic rate below T_{lc} is due to the activation of heat production pathways. Thus, it would mean that *T. brasiliensis* need to increase their heat production at T_a s greater their T_b . In addition, a normothermic $T_b = 29^\circ\text{C}$ could be considered by some to be indicative of an animal in torpor (Barclay et al., 2001; Canale et al., 2012). As an attempt to deal with this contention, we decided to exclude T_b s $< 30^\circ\text{C}$ and recalculated a normothermic $T_b = 32.2^\circ\text{C}$ as the mean of all T_b s $\leq T_{lc}$. It is also noteworthy that because Downs et al. (2012) did not report a normothermic T_b for the *Epomophorus wahlbergi* in their study, we used the normothermic $T_b = 35^\circ\text{C}$ reported for wild caught individuals by Minnaar et al. (2014). Unlike before, the thermolability of the bats in Downs et al. (2012), in combination with the fact that the authors reported mean values, prevented us from calculating a normothermic T_b from the original data.

Results

Phylogenetic signal and body mass

We found evidence of a phylogenetic signal, with significant K -statistic ($p < 0.05$) tests observed for M_b , T_b , T_b-T_{lc} in the overall species data and for T_{uc} in homeothermic species (Table 4-2). The K -value for M_b in the overall data was one, whereas the K -values for T_b and T_b-T_{lc} were less than one. We found a K -value greater than one for T_{uc} in homeotherms (Table 4-2). The influence of phylogeny on the dataset was supported by OLS and PGLS regression analyses. The OLS models showed that M_b has a significant relationship with T_b ($F_{1, 27} = 4.53$, $p = 0.043$) and T_b-T_{lc} ($F_{1, 27} = 4.29$, $p = 0.048$) in the overall dataset, as well as with T_{uc} of homeothermic bats ($F_{1, 9} = 5.49$, $p = 0.044$), but PGLS models showed that the effect of M_b became negligible once corrected for phylogeny. Thus, even though heterothermic bats ($M_b = 24.2 \pm 4.3g$, $n = 18$) were smaller than homeothermic bats ($M_b = 61.2 \pm 16.8g$, $n = 11$) (Table 4-2), once phylogeny was considered, M_b had no effect.

Normothermic body temperature and thermal limits

Heterothermic species had a greater range in normothermic T_b than homeothermic species (Table 4-1, Fig. 4-4), and the mean normothermic T_b for heterothermic species ($T_b = 33.9 \pm 0.5^\circ C$) was significantly lower than that of homeothermic species ($T_b = 36.5 \pm 0.4^\circ C$; $p < 0.01$, Table 4-2). By contrast, the two groups of bats had similar T_{lc} s (heterothermic spp. = $28.9 \pm 0.6^\circ C$ vs homeothermic spp. = $29.0 \pm 1.0^\circ C$) and T_{uc} s (heterothermic spp. = $35.1 \pm 0.9^\circ C$ vs homeothermic spp. = $35.0 \pm 0.7^\circ C$).

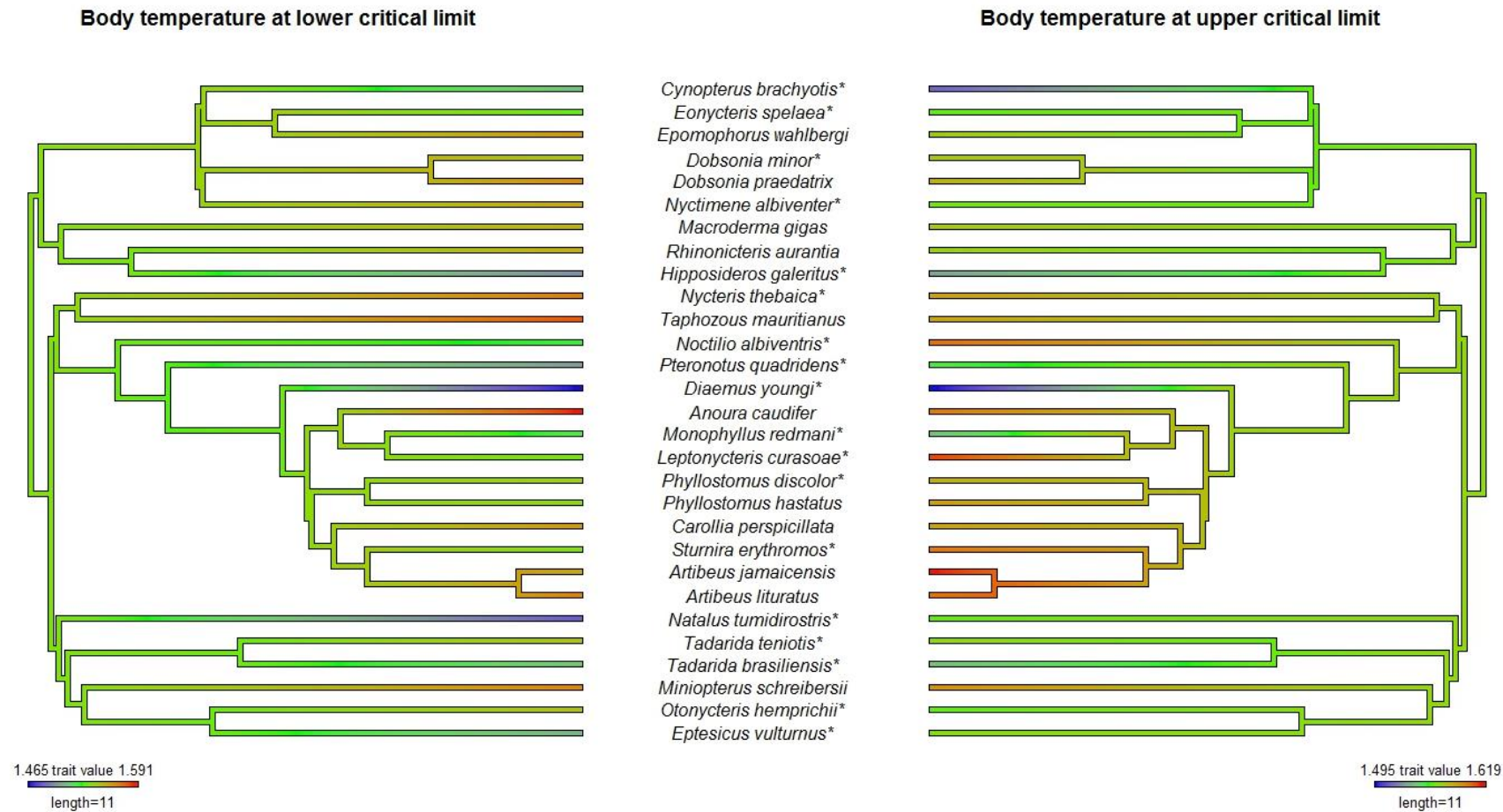


Figure 4-4: The phylogenetic relationship of bats used in the study and their relative body temperatures at the lower (left tree) and upper (right tree) critical limit of thermoneutrality. The phylogenetic tree is based on the phylogeny published by Shi and Rabosky (2015). The T_b range is plotted on a log scale and color coded from low (blue) to high (red). * indicates heterothermic species.

Table 4-1: The body mass (M_b), normothermic body temperature (T_b), lower critical temperature (T_{lc}), upper critical temperature (T_{uc}), body temperature at the lower critical temperature (T_b at T_{lc}), body temperature at the upper critical temperature (T_b at T_{uc}), the difference in body temperature between the lower and upper critical temperatures (ΔT_b), and the species' thermoregulatory classification for the bat species' used in the study. Source indicates the original article from which the raw data were obtained.

Species	M_b (g)	T_b (°C)	T_{lc} (°C)	T_{uc} (°C)	T_b at T_{lc} (°C)	T_b at T_{uc} (°C)	ΔT_b (°C)	Heterothermy	Source
<i>Anoura caudifer</i>	11.5	36.5	33.5	35.0	39.0	40.1	1.1	N	1
<i>Artibeus jamaicensis</i>	45.2	36.4	25.3	36.0	36.3	41.6	5.3	N	1
<i>Artibeus lituratus</i>	70.1	37.3	23.9	35.9	37.4	40.3	2.9	N	1
<i>Carollia perspicillata</i>	14.9	36.4	26.4	35.5	36.8	38.9	2.1	N	1
<i>Cynopterus brachyotis</i>	31.9	32.3	29.0	30.2	32.4	32.6	0.2	Y	2
<i>Diaemus youngi</i>	36.6	31.3	24.4	27.3	29.2	31.3	2.1	Y	1
<i>Dobsonia minor</i>	73.7	36.5	26.7	34.5	35.4	37.8	2.4	Y	3
<i>Dobsonia praedatrix</i>	179.5	37.1	29.6	32.6	37.1	38.6	1.5	N	3
<i>Eonycteris spelaea</i>	51.6	34.0	26.9	33.4	34.1	36.5	2.4	Y	4
<i>Epomophorus wahlbergi</i>	115.0	35.0 ^ψ	25.0	30.0	36.8	37.7	0.9	N	5
<i>Eptesicus vulturnus</i>	4.0	33.3	28.1	37.5	32.12	37.0	4.9	Y	6
<i>Hipposideros galeritus</i>	8.5	31.9	29.1	31.1	31.3	34.0	2.7	Y	4
<i>Leptonycteris curasoae</i>	24.0	35.3	28.0	40.0	34.4	41.2	6.8	Y	7
<i>Macroderma gigas</i>	107.2	35.6	30.0	35.0	36.0	37.8	1.8	N	8
<i>Miniopterus schreibersii</i>	10.9	37.7	32.5	37.5	37.3	39.5	2.2	N	8
<i>Monophyllus redmani</i>	8.8	34.0	32.0	36.0	33.3	34.7	1.4	Y	9

<i>Natalus tumidirostris</i>	5.3	32.2	25.5	35.1	30.2	36.5	6.3	Y	10
<i>Noctilio albiventris</i>	27.0	32.0	30.0	40.0	33.6	40.3	6.7	Y	1
<i>Nycteris thebaica</i>	11.7	38.6	26.2	33.6	37.5	39.0	1.5	Y	11
<i>Nyctimene albiventer</i>	30.9	35.9	28.5	33.5	36.4	36.7	0.3	Y	3
<i>Otonycteris hemprichii</i>	25.0	35.6	33.6	35.0	35.6	36.5	0.9	Y	12
<i>Phyllostomus discolor</i>	33.5	34.6	31.2	38.0	35.1	38.6	3.5	Y	1
<i>Phyllostomus hastatus</i>	84.2	34.7	31.3	36.0	34.8	39.1	4.3	N	1
<i>Pteronotus quadridens</i>	4.8	31.0	30.0	42.0	31.6	35.7	4.1	Y	9
<i>Rhinonictis aurantia</i>	8.3	36.1	32.5	37.5	36.1	37.5	1.4	N	8
<i>Sturnira erythromos</i>	15.9	34.4	28.0	36.7	34.5	40.2	5.7	Y	13
<i>Tadarida brasiliensis</i>	11.0	32.2*	30.8	33.4	32.5	34.8	2.3	Y	13
<i>Tadarida teniotis</i>	32.0	35.1	32.0	35.0	35.5	37.2	1.7	Y	12
<i>Taphozous mauritanus</i>	26.2	38.7	29.0	34.2	38.3	38.9	0.6	N	11

Note:

^ψ T_b obtained from Minnaar et al. (2014)

* T_b recalculated from original source

1 - McNab (1969); 2 – data from Chapter 3, 3 - McNab and Bonaccorso (2001), 4 - McNab (1989), 5 - Downs et al. (2012), 6 - Willis et al. (2005), 7 - Arends et al. (1995), 8 - Baudinette et al. (2000), 9 - Rodríguez-Durán (1995), 10 - Genoud et al. (1990), 11 - Cory Toussaint and McKechnie (2012), 12 - Marom et al. (2006), 13 - Soriano et al. (2002)

Body temperature and evaporative water loss at thermal limits

As with normothermic T_b , there was a greater range in T_b at both T_{lc} and T_{uc} for heterothermic species (Table 4-1). Heterotherms maintained significantly lower T_b s at T_{lc} (T_b at T_{lc} : heterothermic spp. = $33.6 \pm 0.5^\circ\text{C}$, homothermic spp. = $36.9 \pm 0.3^\circ\text{C}$, $p < 0.05$) (Fig. 4-5a) and T_{uc} (T_b at T_{uc} : heterothermic spp. = $36.7 \pm 0.6^\circ\text{C}$ vs homothermic spp. $39.1 \pm 0.4^\circ\text{C}$) (Fig. 4-5b). However, the two groups displayed similar increases in T_b between T_{lc} and T_{uc} (ΔT_b : heterothermic spp. = $3.1 \pm 0.5^\circ\text{C}$ vs homeothermic spp. = $2.2 \pm 0.4^\circ\text{C}$). The fact that the mean T_b at T_{lc} was almost identical to the mean normothermic T_b provides validation for the technique that we used to estimate T_b .

There was no significant difference in mean EWL at T_{lc} or T_{uc} between heterothermic species ($n = 5$, EWL at $T_{lc} = 2.0 \pm 1.2 \text{ mg H}_2\text{O} \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$, EWL at $T_{uc} = 2.3 \pm 1.2 \text{ mg H}_2\text{O} \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$) and homeothermic species ($n = 5$, EWL at $T_{lc} = 5.9 \pm 2.9 \text{ mg H}_2\text{O} \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$, EWL at $T_{uc} = 7.5 \pm 3.6 \text{ mg H}_2\text{O} \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$), even if data from two obvious outliers (*Cynopterus brachyotis* and *Rhinonictis aurantia*) were excluded (Table 4-3).

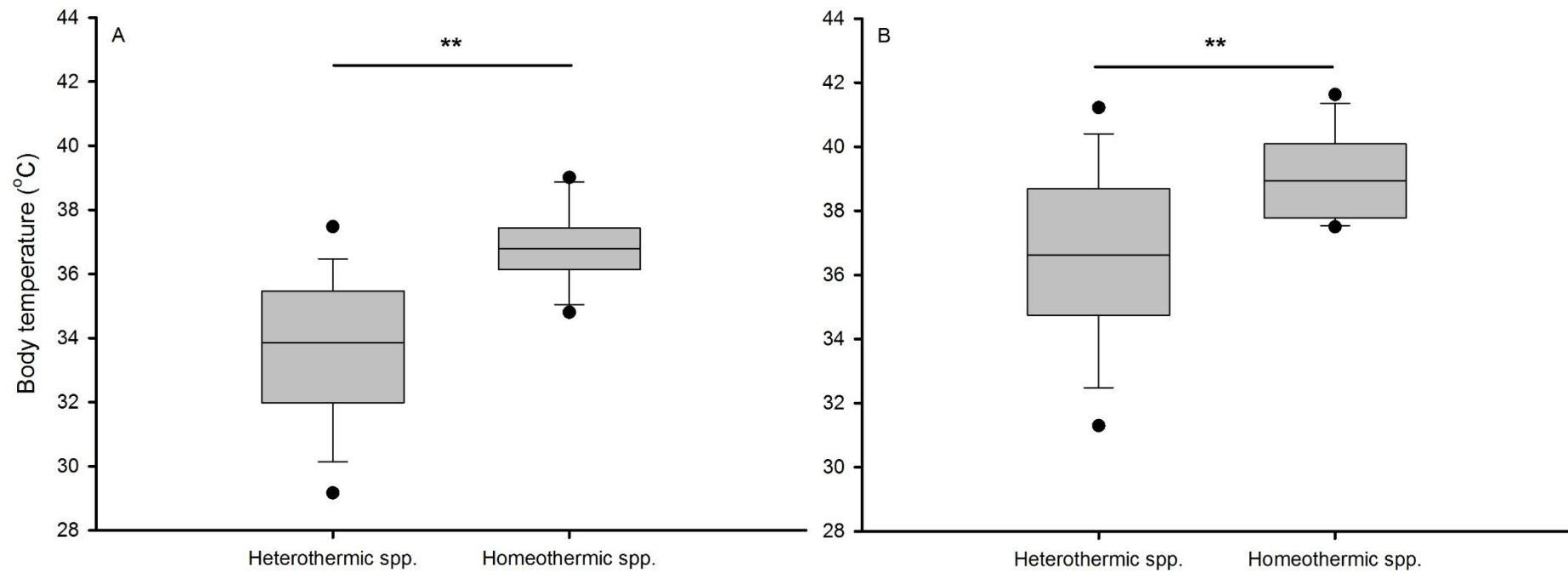


Figure 4-5: Box-and-whisker plots of the calculated body temperatures for the heterothermic (n = 18) and homeothermic (n = 11) bats species used in the study at a) the lower critical limit and b) the upper critical limit. The horizontal line in each box is the median value, the grey boxes define the 25th-75th percentiles, the whiskers define 10th-90th percentiles and black circles represent values beyond those percentiles.

Table 4-2: Summary statistics for Blomberg et al. 2003's *K*-statistic tests for phylogenetic signal in our dataset and the phylogenetic Analysis of Variance (phyloANOVA) tests for comparisons of various physiological variables between heterothermic and homeothermic bat species. M_b = body mass, T_b = normothermic body temperature, $T_b - T_{lc}$ = the measure of T_{lc} expressed as the difference between body temperature and lower critical temperature, T_{uc} = the upper critical temperature, T_b at T_{lc} = the calculated body temperature at the lower critical temperature, T_b at T_{uc} = the calculated body temperature at the upper critical temperature and ΔT_b = the difference in body temperature between the thermal limits. All spp. indicates that all 29 species were included, homeothermic spp. indicates that a subset of only homeothermic species were used ($n = 11$), and heterothermic spp. indicates that a subset of only heterothermic species were used ($n = 18$). * $p < 0.05$, ** $p \leq 0.01$, non-significant values are not reported.

<i>K</i> -values							
Species	M_b	T_b	$T_{lc} (T_b - T_{lc})$	T_{uc}	T_b at T_{lc}	T_b at T_{uc}	ΔT_b
All spp.	1.002**	0.861*	0.936*	-	-	-	-
Homeothermic spp.	-	-	-	1.218**	-	-	-
Heterothermic spp.	-	-	-	-	-	-	-

phyloANOVA comparisons of heterothermic spp. versus homeothermic spp.			
Physiological variable	<i>F</i>	<i>p</i>	
M_b	6.862	0.007	
T_b	14.637	0.001	
$T_{lc} (T_b - T_{lc})$			
T_{uc}			
T_b at T_{lc}	21.039	0.001	
T_b at T_{uc}	7.777	0.004	
ΔT_b			

Table 4-3: The calculated mean evaporative water loss values at the respective thermal limits for each species where data was available.

	Evaporative water loss (mg H ₂ O.g ⁻¹ hr ⁻¹)		
	At T _{lc}	At T _{uc}	Source
<u>Heterothermic species</u>			
<i>Cynopterus brachyotis</i>	0.001	0.002	2
<i>Noctilio albiventris</i>	2.96	3.78	1
<i>Nycteris thebaica</i>	7.67	9.09	11
<i>Otonycteris hemprichii</i>	16.38	20.82	12
<i>Tadarida teniotis</i>	2.62	4.00	12
<u>Homeothermic species</u>			
<i>Epomophorus wahlbergi</i>	6.53	7.06	5
<i>Macroderma gigas</i>	0.11	0.13	8
<i>Miniopterus schreibersii</i>	0.66	1.00	8
<i>Rhinonictis aurantia</i>	1.32	1.49	8
<i>Taphozous mauritanus</i>	1.22	1.62	11

The codes for the source of the information correspond to those in Table 4-1.

Discussion

Understanding how endotherms will respond to global warming has been a recent focal point for research but, developing accurate predictive models is limited both by the availability of data as well as our ability to interpret it (Wolf et al., 2017). Evaluating a species' vulnerability to heat stress, including the intraspecific variation due to phenotypic flexibility is crucial, as heat tolerance provides a rigid limit in its response (Canale and Henry, 2010; Sherwood and Huber, 2010; Boyles et al., 2011; Huey et al., 2012). Here, we tested whether heterotherms can potentially reduce the effects of heat stress through putative hyperthermic torpor. We developed three *a priori* model predictions that would each support our contention for the ability of hyperthermic torpor.

The results do not support our first prediction (Fig 4-3a) that heterotherms would maintain a similar T_b and rate of EWL to homeotherms at T_{uc} , but that T_{uc} would be higher in heterotherms. Likewise, the results do not support our third prediction (Fig 4-3c) that heterotherms would maintain a similar T_b and T_{uc} to homeotherms, but that the rate of EWL would be lower in heterotherms. However, the results do support our second prediction (Fig. 4-3b) that heterotherms would maintain a lower T_b at a similar T_{uc} to homeotherms, but not require additional investment in evaporative cooling to do so. We also found that heterothermic bats maintained lower normothermic T_{bs} than homeothermic bats. Given their low T_{bs} and thus smaller thermal gradient, we expected heterotherms to have a relatively low T_{uc} (Schmidt-Nielsen, 1997; Tattersall et al., 2012). Instead, T_{uc} was ca. 1.2°C higher than normothermic T_b . Consequently, because the heat balance favored heat storage in heterothermic bats, they, by comparison to homeothermic bats, should have displayed higher T_{bs} at T_{uc} relative to their T_{bs} at T_{lc} i.e. ΔT_b , higher absolute T_{bs} or a higher rate of evaporative cooling to reduce heat storage (Sherwood and Huber, 2010; Cory Toussaint and McKechnie, 2012). None of these were observed. We therefore argue that heterothermic bats alter their heat balance through metabolic down-regulation i.e. reducing H_{MR} (see equation 3); which results in an extension of the upper threshold of TNZ. We argue that the reduced heat storage in combination with reduced heat production allowed them to thermoconform at high T_a which presumably negated the need for additional evaporative cooling (*sensu* Bondarenko et al., 2014).

To support the claim that metabolic down-regulation allows heterotherms to maintain lower T_{bs} and reduce evaporative water loss, we plotted T_b as a function of T_a , for both groups of bats. To eliminate potential sample size biases, we determined a mean T_b per T_a for each species and used those values to determine the mean T_b per T_a across species (Fig. 4-6). The interspecific mean T_b plot supports the argument that heterothermic bats maintain lower T_{bs} than homeothermic bats, especially at high T_a s. The plot also shows that heterothermic bats

begin to thermoconform at T_a s that are similar to or slightly higher than normothermic T_b . The benefit of thermoconforming at relatively moderate T_a s is that it may conserve body water for use at more extreme T_a s to maintain $T_b < T_a$; as is evident at $T_a = 40 - 42^\circ\text{C}$. Unfortunately, we do not have sufficient data to determine a reliable estimate of water use for either group at the upper T_a range ($T_a > 36^\circ\text{C}$) as this would have provided empirical support. Nonetheless, the question remains, how do heterothermic bats achieve low T_b s at high T_a s and, as mentioned earlier, why do some bats not display increases in metabolism due to Arrhenius effects at $T_a \geq T_b$?

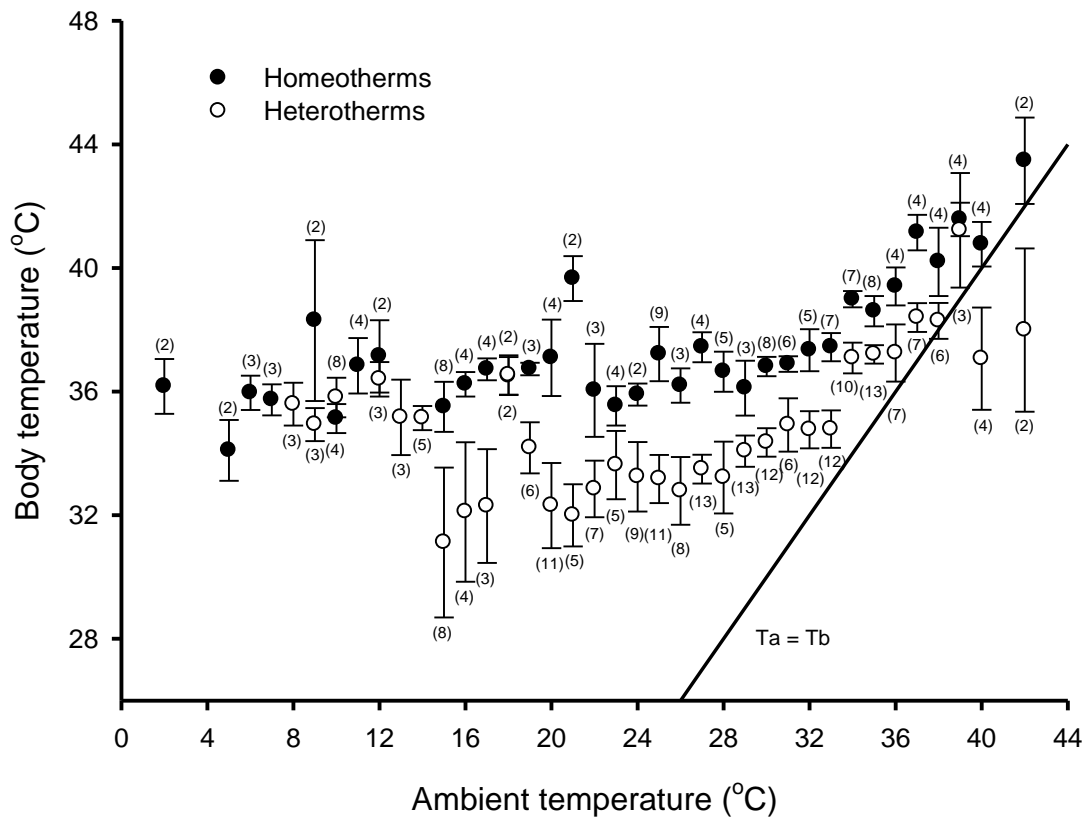


Figure 4-6: Mean \pm standard error of homeothermic and heterothermic bat body temperatures at a range of ambient temperatures. The numbers above and below the plots represent the sample size (n) of homeothermic species and heterothermic species per temperature respectively. Only points with $n > 2$ species are reported.

The characteristic metabolic down-regulation exhibited by heterotherms at low T_a involves a series of complex interrelated biochemical reactions involving hormonal and extracellular triggers that are not fully understood (van Breukelen and Martin, 2002; Storey et al., 2010; Pan et al., 2014; Tessier et al., 2015; van Breukelen and Martin, 2015). Even so, we do have good insight about the mechanisms of how metabolic suppression is achieved and maintained (Biggar et al., 2015; Tessier et al., 2015; Wu et al., 2015; Zhang et al., 2015). Metabolism is regulated, at the molecular level, by enzyme-mediated reversible phosphorylation of post-translational proteins (Melvin and Andrews, 2009; Storey et al., 2010). For example, a well-known biochemical mechanism used to lower metabolism during bouts of hypothermia involves the inhibition of pyruvate dehydrogenase by pyruvate dehydrogenase kinase, which interrupts glycolysis (Melvin and Andrews, 2009). Another pathway involves the activation of 5' adenosine monophosphate-activated protein kinase (AMPK) that plays a facilitatory role in ATP synthesis and leads to a simultaneous reduction in the overall rate of ATP consumption within the body (Melvin and Andrews, 2009; Storey et al., 2010). Thus, because hypometabolism is achieved by an inhibition of the processes that generate ATP and not on temperature dependent processes *per se*, an up-regulation of inhibitory enzymes at high T_a may still reduce metabolism. This process would circumvent Arrhenius effects on metabolism and explain why some bats have extremely high T_{ucS} .

Even though there are several metabolic pathways, AMPK is the master regulator governing energy homeostasis at the cellular level (Hardie et al., 2003; Storey et al., 2010; Mihaylova and Shaw, 2011) and seems the most likely mechanism involved in hyperthermic torpor. Mammalian AMPK is activated in response to stressors that affect the ratio between AMP and ATP, which amongst others, includes heat shock (reviewed by Hardie and Hawley, 2001). It may be that the heat shock response during hyperthermia activates AMPK that then facilitates a metabolic down-regulation. Thus, understanding the link between heat shock and hypometabolism is an integral component to the argument for hyperthermic torpor. Heat shock proteins are, as their name suggests, typically up-regulated in response to heat stress, but their expression and role extends far beyond their eponym (reviewed by Feder and Hofmann, 1999). Indeed, heat shock proteins are up-regulated in heterotherms during episodes of torpor or hibernation even though there is no heat stress (Storey and Storey, 2011; Wu et al., 2015) and this suggests that they may be involved. However, whether heat shock proteins provide the mechanistic link between metabolic down-regulation and high T_{aS} , or whether their expression is just a covariate response, needs to be determined. Currently, there is equivocal evidence that the interaction between AMPK and heat shock proteins may be antagonistic (for example Tsai et al., 2016) or synergistic (for example Wang et al., 2010). Clearly, more

research is needed to evaluate the interaction between these molecules in heterotherms versus homeotherms.

We acknowledge that our analyses are limited by few data and, given our approach, it seems that heterothermic species that are most effective at presumed hyperthermic torpor, such as *M. blainvilli* (Rodríguez-Durán, 1995), *M. condylurus* (Maloney et al., 1999), *P. quadridens* (Rodríguez-Durán, 1995), *Sturnia lilium* (McNab, 1969) and *Tonatia bidens* (McNab, 1969), as well as the potential heterotherm *Sauromys petrophilus* (Cory Toussaint and McKechnie, 2012), among others, were excluded. We need data from these species to ascertain their respective T_{uc} s and redo our analyses. It would also be worthwhile for future studies to attempt direct determination of metabolic down-regulation and the simultaneous measurement of the concentration of heat shock proteins in combination with other potential marker enzymes such as AMPK or pyruvate dehydrogenase kinase. We predict that one or more of these enzymes would be upregulated in heterotherms at high T_a , and that they would lead to a hypometabolic response. We further suggest that such studies also focus on marsupials as hyperthermic torpor has been claimed to occur in the grey short-tailed opossum (*Monodelphis domestica*; Busse et al., 2014) and eastern pygmy-possum (*Cercartetus nanus*; Song et al., 1997) or possibly rodents, due to the claim of hypothermic torpor in spiny mice (*Acomys russatus*, Grimpot et al., 2013). In addition, more thermoregulatory data that include EWL could help explain the variation in the onset of evaporative cooling and aid our understanding of the water conservation benefits of hyperthermic torpor.

Conflict of interest statement

The authors declare no financial, or other, competing interests.

Author contributions

S.W. and B.G.L conceived the study. S.W. designed the study, developed the model predictions, performed the data collection, data analyses and drafted the manuscript. B.G.L. provided advice about the data analyses as well as contributed to and approved the manuscript.

Acknowledgements

We thank Andrew McKechnie and Mark Brigham for their valuable comments on the initial drafts of the manuscript. We also thank Andrew McKechnie for providing us with raw data from the studies that he co-authored.

Funding

This work was supported by the South African National Research Foundation, the GreenMatter fellowship in conjunction with their partners and the Gay Langmuir Scholarship.

Literature cited

- Adams, M., Reardon, T.R., Baverstock, P.R., and Watts, C.H.S. (1988). Electrophoretic resolution of species boundaries in Australian Microchiroptera. IV. The Molossidae (Chiroptera). *Aust. J. Biol. Sci.* 41(3), 315-326. doi: <https://doi.org/10.1071/BI9880315>.
- Angilletta Jr, M.J., Cooper, B.S., Schuler, M.S., and Boyles, J.G. (2010). The evolution of thermal physiology in endotherms. *Front. Biosci.* 2, 861-881.
- Arends, A., Bonaccorso, F.J., and Genoud, M. (1995). Basal rates of metabolism of nectarivorous bats (Phyllostomidae) from a semiarid thorn forest in Venezuela. *J. Mammal.* 76(3), 947-956. doi: 10.2307/1382765.
- Arrhenius, S. (1915). *Quantitative laws in biological chemistry*. London: Bell and Sons Ltd.
- Barclay, R.M.R., Lausen, C.L., and Hollis, L. (2001). What's hot and what's not: defining torpor in free-ranging birds and mammals. *Can. J. Zool.* 79(10), 1885-1890. doi: 10.1139/z01-138.
- Bartels, W., Law, B.S., and Geiser, F. (1998). Daily torpor and energetics in a tropical mammal, the northern blossom-bat *Macroglossus minimus* (Megachiroptera). *J. Comp. Physiol. B.* 168(3), 233-239. doi: 10.1007/s003600050141.
- Baudinette, R.V., Churchill, S.K., Christian, K.A., Nelson, J.E., and Hudson, P.J. (2000). Energy, water balance and the roost microenvironment in three Australian cave-dwelling bats (Microchiroptera). *J. Comp. Physiol. B.* 170(5-6), 439-446. doi: 10.1007/s003600000121.
- Biggar, K.K., Wu, C.-W., Tessier, S.N., Zhang, J., Pifferi, F., Perret, M., et al. (2015). Primate torpor: regulation of stress-activated protein kinases during daily torpor in the gray mouse lemur, *Microcebus murinus*. *Genomics Proteomics Bioinformatics* 13(2), 81-90. doi: <https://doi.org/10.1016/j.gpb.2015.03.002>.
- Blomberg, S.P., Garland Jr, T., and Ives, A.R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* 57(4), 717-745. doi: 10.1111/j.0014-3820.2003.tb00285.x.

- Bondarenko, A., Körtner, G., and Geiser, F. (2014). Hot bats: extreme thermal tolerance in a desert heat wave. *Naturwissenschaften* 101(8), 679-685. doi: 10.1007/s00114-014-1202-2.
- Boyles, J.G., Seebacher, F., Smit, B., and McKechnie, A.E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* 51, 676-690.
- Busse, S., Lutter, D., Heldmaier, G., Jastroch, M., and Meyer, C.W. (2014). Torpor at high ambient temperature in a neotropical didelphid, the grey short-tailed opossum (*Monodelphis domestica*). *Naturwissenschaften* 101(11), 1003-1006. doi: 10.1007/s00114-014-1226-7.
- Canale, C.I., and Henry, P.Y. (2010). Adaptive phenotypic plasticity and resilience of vertebrates to increasing climatic unpredictability. *Clim. Res.* 43(1-2), 135-147. doi: 10.3354/cr00897.
- Canale, C.I., Levesque, D.L., and Lovegrove, B.G. (2012). "Tropical heterothermy: does the exception prove the rule or force a re-definition?," in *Living in a seasonal world: thermoregulatory and metabolic adaptations*, eds. T. Ruf, C. Bieber, W. Arnold & E. Millesi. (Heidelberg, Berlin: Springer), 29-40.
- Clarke, A., and Pörtner, H.-O. (2010). Temperature, metabolic power and the evolution of endothermy. *Biol. Rev.* 85(4), 703-727. doi: 10.1111/j.1469-185X.2010.00122.x.
- Clarke, A., Rothery, P., and Isaac, N.J.B. (2010). Scaling of basal metabolic rate with body mass and temperature in mammals. *J. Anim. Ecology* 79, 610-619.
- Cooper, C.E., and Withers, P.C. (2012). Does season or captivity influence the physiology of an endangered marsupial, the numbat (*Myrmecobius fasciatus*)? *J. Mammal.* 93(3), 771-777. doi: 10.1644/11-MAMM-A-084.2.
- Cory Toussaint, D., and McKechnie, A.E. (2012). Interspecific variation in thermoregulation among three sympatric bats inhabiting a hot, semi-arid environment. *J. Comp. Physiol. B.* 182(8), 1129-1140. doi: 10.1007/s00360-012-0683-6.
- Dausmann, K.H. (2014). Flexible patterns in energy savings: heterothermy in primates. *J. Zool.* 292(2), 101-111. doi: 10.1111/jzo.12104.
- Doty, A.C., Stawski, C., Law, B.S., and Geiser, F. (2016). Post-wildfire physiological ecology of an Australian microbat. *J. Comp. Physiol. B.* 186(7), 937-946. doi: 10.1007/s00360-016-1003-3.

- Downs, C.T., Zungu, M.M., and Brown, M. (2012). Seasonal effects on thermoregulatory abilities of the Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) in KwaZulu-Natal, South Africa. *J. Therm. Biol.* 37(2), 144-150. doi: <http://dx.doi.org/10.1016/j.jtherbio.2011.12.003>.
- Feder, M.E., and Hofmann, G.E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu. Rev. Physiol.* 61(1), 243-282. doi: [doi:10.1146/annurev.physiol.61.1.243](https://doi.org/10.1146/annurev.physiol.61.1.243).
- Fristoe, T.S., Burger, J.R., Balk, M.A., Khaliq, I., Hof, C., and Brown, J.H. (2015). Metabolic heat production and thermal conductance are mass-independent adaptations to thermal environment in birds and mammals. *Proc. Natl. Acad. Sci. U. S. A.* 112(52), 15934-15939. doi: [10.1073/pnas.1521662112](https://doi.org/10.1073/pnas.1521662112).
- Fuller, A., Dawson, T., Helmuth, B., Hetem, R.S., Mitchell, D., and Maloney, S.K. (2010). Physiological mechanisms in coping with climate change. *Physiol. Biochem. Zool.* 83(5), 713-720. doi: [10.1086/652242](https://doi.org/10.1086/652242).
- Fuller, A., Mitchell, D., Maloney, S.K., and Hetem, R.S. (2016). Towards a mechanistic understanding of the responses of large terrestrial mammals to heat and aridity associated with climate change. *Clim. Chang. Responses* 3:10(1). doi: [10.1186/s40665-016-0024-1](https://doi.org/10.1186/s40665-016-0024-1).
- Geiser, F. (1988). Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? *J. Comp. Physiol. B.* 158(1), 25-37. doi: [10.1007/bf00692726](https://doi.org/10.1007/bf00692726).
- Geiser, F., and Brigham, R.M. (2000). Torpor, thermal biology, and energetics in Australian long-eared bats (*Nyctophilus*). *J. Comp. Physiol. B.* 170(2), 153-162. doi: [10.1007/s0036000050270](https://doi.org/10.1007/s0036000050270).
- Geiser, F., and Brigham, R.M. (2012). "The other functions of torpor," in *Living in a seasonal world: thermoregulatory and metabolic adaptations*, eds. T. Ruf, C. Bieber, W. Arnold & E. Millesi. (Heidelberg, Berlin: Springer), 109-121.
- Geiser, F., and Turbill, C. (2009). Hibernation and daily torpor minimize mammalian extinctions. *Naturwissenschaften* 96(10), 1235-1240. doi: [10.1007/s00114-009-0583-0](https://doi.org/10.1007/s00114-009-0583-0).
- Genoud, M., Bonaccorso, F.J., and Anends, A. (1990). Rate of metabolism and temperature regulation in two small tropical insectivorous bats (*Peropteryx macrotis* and *Natalus tumidirostris*). *Comp. Biochem. Physiol. A.* 97(2), 229-234. doi: [http://dx.doi.org/10.1016/0300-9629\(90\)90177-T](http://dx.doi.org/10.1016/0300-9629(90)90177-T).

- Grimpo, K., Legler, K., Heldmaier, G., and Exner, C. (2013). That's hot: golden spiny mice display torpor even at high ambient temperatures. *J. Comp. Physiol. B.* 183(4), 567-581. doi: 10.1007/s00360-012-0721-4.
- Hackshaw, A. (2009). "Statistical formulae for calculating some 95% confidence intervals," in *A Concise Guide to Clinical Trials*. (West Sussex, UK: Wiley-Blackwell), 205-207.
- Hallam, S.L., and Mzilikazi, N. (2011). Heterothermy in the southern African hedgehog, *Atelerix frontalis*. *J. Comp. Physiol. B.* 181(3), 437-445. doi: 10.1007/s00360-010-0531-5.
- Hardie, D.G., and Hawley, S.A. (2001). AMP-activated protein kinase: the energy charge hypothesis revisited. *BioEssays* 23(12), 1112-1119. doi: 10.1002/bies.10009.
- Hardie, D.G., Scott, J.W., Pan, D.A., and Hudson, E.R. (2003). Management of cellular energy by the AMP-activated protein kinase system. *FEBS Letters* 546(1), 113-120. doi: [http://dx.doi.org/10.1016/S0014-5793\(03\)00560-X](http://dx.doi.org/10.1016/S0014-5793(03)00560-X).
- Heldmaier, G. (Year). "Seasonal acclimatization of energy requirements in mammals: functional significance of body weight control, hypothermia, torpor and hibernation", in: *Energy Transformations in cells and organisms: proceedings of the 10th conference of the European society for comparative physiology and biochemistry*, eds. W. Wieser & E. Gnaiger: Thieme), 130-139.
- Hetem, R.S., Fuller, A., Maloney, S.K., and Mitchell, D. (2014). Responses of large mammals to climate change. *Temperature* 1(2), 115-127. doi: 10.4161/temp.29651.
- Hosken, D.J., and Withers, P.C. (1997). Temperature regulation and metabolism of an Australian bat, *Chalinolobus gouldii* (Chiroptera: Vespertilionidae) when euthermic and torpid. *J. Comp. Physiol. B.* 167(1), 71-80. doi: 10.1007/s003600050049.
- Hosken, D.J., and Withers, P.C. (1999). Metabolic physiology of euthermic and torpid lesser long-eared bats, *Nyctophilus geoffroyi* (Chiroptera: Vespertilionidae). *J. Mammal.* 80(1), 42-52. doi: 10.2307/1383206.
- Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A., Jess, M., and Williams, S.E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 367(1596), 1665-1679. doi: 10.1098/rstb.2012.0005.
- Huwaldt, J.A., and Stenhorst, S. (2015). *Plot Digitizer* [Online]. Available: <http://plotdigitizer.sourceforge.net/> [Accessed].

- IUPS Thermal Commission (2003). Glossary of terms for thermal physiology. *J. Therm. Biol.* 28(1), 75-106. doi: [http://dx.doi.org/10.1016/S0306-4565\(02\)00055-4](http://dx.doi.org/10.1016/S0306-4565(02)00055-4).
- Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., et al. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26(11), 1463-1464. doi: 10.1093/bioinformatics/btq166.
- Khaliq, I., Hof, C., Prinzinger, R., Böhning-Gaese, K., and Pfenninger, M. (2014). Global variation in thermal tolerances and vulnerability of endotherms to climate change. *Proc. R. Soc. B.* 281:1789. doi: 10.1098/rspb.2014.1097.
- Lloyd, J., and Taylor, J.A. (1994). On the temperature dependence of soil respiration. *Funct. Ecol.* 8(3), 315-323. doi: 10.2307/2389824.
- Lovegrove, B.G. (2003). The influence of climate on the basal metabolic rate of small mammals: a slow-fast metabolic continuum. *J. Comp. Physiol. B.* 173(2), 87-112. doi: 10.1007/s00360-002-0309-5.
- Lovegrove, B.G. (2005). Seasonal thermoregulatory responses in mammals. *J. Comp. Physiol. B.* 175(4), 231-247. doi: 10.1007/s00360-005-0477-1.
- Lovegrove, B.G. (2012). The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol. Rev.* 87(1), 128-162. doi: 10.1111/j.1469-185X.2011.00188.x.
- Lovegrove, B.G. (2017). A phenology of the evolution of endothermy in birds and mammals. *Biol. Rev.* 92, 1213-1240. doi: 10.1111/brv.12280.
- Lovegrove, B.G., Canale, C., Levesque, D.L., Fluch, G., Rehakova-Petru, M., and Ruf, T. (2014a). Are tropical small mammals physiologically vulnerable to arrhenius effects and climate change? *Physiol. Biochem. Zool.* 87(1), 30-45. doi: 10.1086/673313.
- Lovegrove, B.G., Heldmaier, G., and Knight, M. (1991a). Seasonal and circadian energetic patterns in an arboreal rodent, *Thallomys paedulus*, and a burrow-dwelling rodent, *Aethomys namaquensis*, from the kalahari desert. *J Therm Biol* 16(4), 199-209. doi: 10.1016/0306-4565(91)90026-x.
- Lovegrove, B.G., Heldmaier, G., and Ruf, T. (1991b). Perspectives of endothermy revisited - the endothermic temperature-range. *J Therm Biol* 16(4), 185-197. doi: 10.1016/0306-4565(91)90025-w.
- Lovegrove, B.G., Lobban, K.D., and Levesque, D.L. (2014b). Mammal survival at the Cretaceous–Palaeogene boundary: metabolic homeostasis in prolonged tropical hibernation in tenrecs. *Proc. R. Soc. B.* 281:20141304. doi: 10.1098/rspb.2014.1304.

- Maloney, S.K., Bronner, G.N., and Buffenstein, R. (1999). Thermoregulation in the Angolan free-tailed bat *Mops condylurus*: a small mammal that uses hot roosts. *Physiol. Biochem. Zool.* 72(4), 385-396. doi: 10.1086/316677.
- Marom, S., Korine, C., Wojciechowski, M., Tracy, C.R., and Pinshow, B. (2006). Energy metabolism and evaporative water loss in the European free-tailed bat and Hemprich's long-eared bat (Microchiroptera): species sympatric in the Negev desert. *Physiol. Biochem. Zool.* 79(5), 944-956. doi: 10.1086/505999.
- McCain, C.M., and King, S.R.B. (2014). Body size and activity times mediate mammalian responses to climate change. *Glob. Chang. Biol.* 20(6), 1760-1769. doi: 10.1111/gcb.12499.
- McKechnie, A.E., and Mzilikazi, N. (2011). Heterothermy in Afrotropical mammals and birds: a review. *Integr. Comp. Biol.* 51(3), 349-363. doi: 10.1093/icb/icr035.
- McNab, B.K. (1969). The economics of temperature regulation in neotropical bats. *Comp. Biochem. Physiol.* 31(2), 227-268.
- McNab, B.K. (1980). On estimating thermal conductance in endotherms. *Physiol. Zool.* 53(2), 145-156. doi: 10.1086/physzool.53.2.30152577.
- McNab, B.K. (1989). Temperature regulation and rate of metabolism in 3 Bornean bats. *J. Mammal.* 70(1), 153-161. doi: 10.2307/1381678.
- McNab, B.K., and Bonaccorso, F.J. (2001). The metabolism of New Guinean pteropodid bats. *J. Comp. Physiol. B.* 171(3), 201-214. doi: 10.1007/s003600000163.
- Melvin, R.G., and Andrews, M.T. (2009). Torpor induction in mammals: recent discoveries fueling new ideas. *Trends Endocrinol. Metab.* 20(10), 490-498. doi: 10.1016/j.tem.2009.09.005.
- Mihaylova, M.M., and Shaw, R.J. (2011). The AMP-activated protein kinase (AMPK) signaling pathway coordinates cell growth, autophagy, & metabolism. *Nat. Cell. Biol.* 13(9), 1016-1023. doi: 10.1038/ncb2329.
- Minnaar, I.A., Bennett, N.C., Chimimba, C.T., and McKechnie, A.E. (2014). Summit metabolism and metabolic expansibility in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*): seasonal acclimatisation and effects of captivity. *J. Exp. Biol.* 217, 1363-1369. doi: 10.1242/jeb.098400.
- Muggeo, V.M. (2008). "Segmented: an R package to fit regression models with broken-line relationships". *R News*, 8/1/, 20-25).

- Nowack, J., Cooper, C.E., and Geiser, F. (2016). Cool echidnas survive the fire. *Proc. R. Soc. B.* 283:20160382. doi: 10.1098/rspb.2016.0382.
- Nowack, J., Rojas, A.D., Körtner, G., and Geiser, F. (2015). Snoozing through the storm: torpor use during a natural disaster. *Sci. Rep.* 5:11243. doi: 10.1038/srep11243.
- Nowack, J., Stawski, C., and Geiser, F. (2017). More functions of torpor and their roles in a changing world. *J. Comp. Physiol. B.* 187(5), 889-897. doi: 10.1007/s00360-017-1100-y.
- O'Mara, M.T., Rikker, S., Wikelski, M., Ter Maat, A., Pollock, H.S., and Dechmann, D.K.N. (2017). Heart rate reveals torpor at high body temperatures in lowland tropical free-tailed bats. *R. Soc. Open Sci.* 4(12). doi: 10.1098/rsos.171359.
- Pan, P., Treat, M.D., and van Breukelen, F. (2014). A systems-level approach to understanding transcriptional regulation by p53 during mammalian hibernation. *J. Exp. Biol.* 217(14), 2489-2498. doi: 10.1242/jeb.103614.
- Paradis, E., Claude, J., and Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20(2), 289-290. doi: 10.1093/bioinformatics/btg412.
- Quintero, I., and Wiens, J.J. (2013). Rates of projected climate change dramatically exceed past rates of climatic niche evolution among vertebrate species. *Ecol. Lett.* 16(8), 1095-1103. doi: 10.1111/ele.12144.
- R Core Team (2017). "R: A language and environment for statistical computing". (Vienna, Austria: R Foundation for Statistical Computing).
- Rahmstorf, S., Cazenave, A., Church, J.A., Hansen, J.E., Keeling, R.F., Parker, D.E., et al. (2007). Recent climate observations compared to projections. *Science* 316:709(5825). doi: 10.1126/science.1136843.
- Revell, L.J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3(2), 217-223. doi: 10.1111/j.2041-210X.2011.00169.x.
- Riek, A., and Geiser, F. (2013). Allometry of thermal variables in mammals: consequences of body size and phylogeny. *Biol. Rev.* 88(3), 564-572. doi: 10.1111/brv.12016.
- Rodríguez-Durán, A. (1995). Metabolic rates and thermal conductance in four species of neotropical bats roosting in hot caves. *Comp. Biochem. Phys. A.* 110(4), 347-355. doi: [http://dx.doi.org/10.1016/0300-9629\(94\)00174-R](http://dx.doi.org/10.1016/0300-9629(94)00174-R).
- Ruf, T., and Geiser, F. (2015). Daily torpor and hibernation in birds and mammals. *Biol. Rev.* 90(3), 891-926. doi: 10.1111/brv.12137.

- Schmidt-Nielsen, K. (1997). *Animal physiology: adaptation and environment*. Cambridge: Cambridge University Press.
- Scholander, P.F., Hock, R., Walters, V., Johnson, F., and Irving, L. (1950). Heat regulation in some arctic and tropical mammals and birds. *Biol. Bull.* 99(2), 237-258.
- Sherwood, S.C., and Huber, M. (2010). An adaptability limit to climate change due to heat stress. *Proc. Natl. Acad. Sci. U. S. A.* 107(21), 9552-9555. doi: 10.1073/pnas.0913352107.
- Shi, J.J., and Rabosky, D.L. (2015). Speciation dynamics during the global radiation of extant bats. *Evolution* 69(6), 1528-1545. doi: 10.1111/evo.12681.
- Song, X., Körtner, G., and Geiser, F. (1997). Thermal relations of metabolic rate reduction in a hibernating marsupial. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 273(6), R2097-R2104.
- Soriano, P.J., Ruiz, A., and Arends, A. (2002). Physiological responses to ambient temperature manipulation by three species of bats from Andean cloud forests. *J. Mammal.* 83(2), 445-457. doi: 10.2307/1383571.
- Stawski, C., Willis, C.K.R., and Geiser, F. (2014). The importance of temporal heterothermy in bats. *J. Zool.* 292(2), 86-100. doi: 10.1111/jzo.12105.
- Storey, K.B., Heldmaier, G., and Rider, M.H. (2010). "Mammalian hibernation: physiology, cell signaling, and gene controls on metabolic rate depression," in *Dormancy and resistance in harsh environments*, eds. E. Lubzens, J. Cerda & M. Clark. (Berlin, Heidelberg: Springer Berlin Heidelberg), 227-252.
- Storey, K.B., and Storey, J.M. (2011). Heat shock proteins and hypometabolism: adaptive strategy for proteome preservation. *Res. Rep. Biol.* 2, 57-68.
- Tattersall, G.J., Sinclair, B.J., Withers, P.C., Fields, P.A., Seebacher, F., Cooper, C.E., et al. (2012). Coping with thermal challenges: Physiological adaptations to environmental temperatures. *Compr. Physiol.* 2, 2151-2202. doi: 10.1002/cphy.c110055.
- Tessier, S.N., Zhang, J., Biggar, K.K., Wu, C.-W., Pifferi, F., Perret, M., et al. (2015). Regulation of the PI3K/AKT pathway and fuel utilization during primate torpor in the gray mouse lemur, *Microcebus murinus*. *Genomics Proteomics Bioinformatics* 13(2), 91-102. doi: <https://doi.org/10.1016/j.gpb.2015.03.006>.
- Tsai, Y.C., Lam, K.K., Peng, Y.J., Lee, Y.M., Yang, C.Y., Tsai, Y.J., et al. (2016). Heat shock protein 70 and AMP-activated protein kinase contribute to 17-DMAG-dependent

- protection against heat stroke. *J. Cell. Mol. Med.* 20(10), 1889-1897. doi: 10.1111/jcmm.12881.
- van Breukelen, F., and Martin, S. (2002). Reversible depression of transcription during hibernation. *J. Comp. Physiol. B.* 172(5), 355-361. doi: 10.1007/s00360-002-0256-1.
- van Breukelen, F., and Martin, S.L. (2015). The hibernation continuum: physiological and molecular aspects of metabolic plasticity in mammals. *Physiology* 30(4), 273-281. doi: 10.1152/physiol.00010.2015.
- Wang, T., Yu, Q., Chen, J., Deng, B., Qian, L., and Le, Y. (2010). PP2A mediated AMPK inhibition promotes HSP70 expression in heat shock response. *PLOS ONE* 5(10), e13096. doi: 10.1371/journal.pone.0013096.
- Willis, C.K.R., Turbill, C., and Geiser, F. (2005). Torpor and thermal energetics in a tiny Australian vespertilionid, the little forest bat (*Vespadelus vulturnus*). *J. Comp. Physiol. B.* 175(7), 479-486. doi: 10.1007/s00360-005-0008-0.
- Withers, P.C. (1992). *Comparative animal physiology*. Orlando, FL: Saunder College Publishing.
- Wolf, B.O., Coe, B.H., Gerson, A.R., and McKechnie, A.E. (2017). Comment on an analysis of endotherm thermal tolerances: systematic errors in data compilation undermine its credibility. *Proc. R. Soc. B.* 284: 20162523.. doi: 10.1098/rspb.2016.2523.
- Wu, C.-W., Biggar, K.K., Zhang, J., Tessier, S.N., Pifferi, F., Perret, M., et al. (2015). Induction of antioxidant and heat shock protein responses during torpor in the gray mouse lemur, *Microcebus murinus*. *Genomics Proteomics Bioinformatics* 13(2), 119-126. doi: <https://doi.org/10.1016/j.gpb.2015.03.004>.
- Zhang, J., Tessier, S.N., Biggar, K.K., Wu, C.-W., Pifferi, F., Perret, M., et al. (2015). Regulation of torpor in the gray mouse lemur: transcriptional and translational controls and role of AMPK signaling. *Genomics Proteomics Bioinformatics* 13(2), 103-110. doi: <https://doi.org/10.1016/j.gpb.2015.03.003>.

Chapter Five – Thesis summary and future recommendations

Vulnerability of tropical mammals to heat stress due to climate change

Understanding how endotherms will respond to global warming has been a recent focal point, but the development of accurate predictive models are limited by the availability of quality data as well as the ability to interpret the data (Wolf et al. 2017). It has been suggested that tropical endotherms are particularly vulnerable to heat stress due to global warming (Lovegrove et al. 2014). Their vulnerability is a consequence of their reduced capacity to dissipate excess body heat owed to a combination of their low metabolism, low body temperatures (T_b), and the warm and humid environment that they inhabit (Tattersall et al. 2012, Lovegrove et al. 2014). Thus, as global warming continues, tropical endotherms will have a diminished capacity to avoid hyperthermia and increased heat storage may lead to lethal hyperthermia. Certainly, the thermoregulatory data presented in the current thesis supports the concern that tropical small mammals that have low T_b s are physiological susceptible to heat stress by the end of this century.

Chapters 2 and 3 show, respectively, that tarsiers (*Cephalopachus bancanus*) and lesser dog-faced fruit bats (*Cynopterus brachyotis*), maintain core T_b s that are lower than previously reported (McNab & Wright 1987, McNab 1989, Lovegrove et al. 2014). As expected, both species started to display increased heat storage as ambient temperature (T_a) approached T_b (Tattersall et al. 2012). Worrisomely, because of their respective low T_b , the onset of heat storage occurred at seemingly moderate T_a in both study species. The laboratory data show that heat dissipation, in a dry atmosphere, was only effective at $T_a \leq 30^\circ\text{C}$ in *C. bancanus* and $T_a \leq 31^\circ\text{C}$ in *C. brachyotis*, above which heat storage became apparent. Thus, due to the high relative humidity conditions of the tropics, there is great concern for many tropical endotherms because they are likely to encounter natural situations that are less favourable for heat dissipation than those used in the laboratory experiments (Tattersall et al. 2012, Lovegrove et al. 2014).

Fortunately, many forest dwellers are likely to experience less intense increases in T_a than what is typically predicted because forest habitats are generally buffered and cooler than the external environment (Jacobs 1988, Karlsson 2000, Tuff et al. 2016). Indeed, the T_a recorded at the two capture sites used in Chapter 3 were fairly stable and only rarely exceeded 30°C . It is therefore likely that even though some species may be physiologically susceptible to heat stress, they may be able to avoid high T_a by exploiting thermal refugia. Unfortunately, land use changes are extensive throughout the tropics and particularly aggressive throughout

Borneo (Gaveau et al. 2016). Typically, these changes in land use lead to habitat fragmentation which creates less hospitable conditions within remaining forest patches because it alters the microclimate within those patches (Turner & Corlett 1996, Laurance 2004, Tuff et al. 2016). Unfortunately, a direct comparison between the T_a records presented in Chapter 2 and Chapter 3 are not possible because they do not correspond to the same time period. Nonetheless, the data suggest that there was a large difference between the thermal stability at the respective sites. Whereas the more disturbed and open capture site used in Chapter 2 frequently displayed amplitudes of ca. 8°C or more in daily temperature, the less disturbed capture sites used in Chapter 3 remained relatively cool and the amplitude in daily temperature was mostly < 6°C. Thus, the data provide some support for the argument that habitat fragmentation alters the microclimate of forests and diminishes their capacity to buffer temperature variability.

The current thesis reaffirms the value of adopting a mechanistic approach when assessing a species response to global warming (Ricklefs & Wikelski 2002, Chevin et al. 2010, Chown et al. 2010, Kearney et al. 2010, Sherwood & Huber 2010, Lovegrove et al. 2014). By incorporating data about the microclimate experienced by the study species with data about their thermoregulatory capacity, as well as including information regarding regional trends in land use practises, the current thesis presents a more holistic understanding of the vulnerability of the study species to global warming and their likely response. Overall, the thesis presents the argument that conservationists must, as a matter of urgency, begin to preemptively prioritize and protect tropical forests to maintain their integrity and value as thermal refugia. Given the susceptibility of tropical endotherms to heat stress, failing to take decisive conservation action could lead to population collapses that undoubtedly will have dire consequences for the ecosystem.

The benefits of adaptive heterothermy during heat stress

Even though *C. bancanus* and *C. brachyotis* started to store heat at similar T_a (30-31°C), heat storage appeared to be less pronounced in *C. brachyotis* because they seemingly thermoconformed at T_a above their thermoneutral zone (TNZ), whereas *C. bancanus* did not. This observation suggests that *C. brachyotis* may have entered torpor in response to heat i.e. entered hyperthermic torpor. Currently, our understanding of thermoregulatory responses to high T_a , that is, at T_a where animals face hyperthermia, is limited. There are only a handful of studies that support the claim of hyperthermic torpor (Song et al. 1997, Grimpot et al. 2013, Bondarenko et al. 2014, Busse et al. 2014), yet many bat species display putative characteristics of hyperthermic torpor. For example, Angolan Free-tailed bats (*Mops condylurus*, T_b = 35.2°C, mass = 23.2g; Maloney et al. 1999), sooty mustached bats

(*Pteronotus quadridens*, $T_b = 31.2^\circ\text{C}$, mass = 4.8g; Rodríguez-Durán 1995) and Antillean ghost-faced bats (*Mormoops blainvilli*, $T_b = 32.0^\circ\text{C}$, mass = 8.6g; Rodríguez-Durán 1995) display extremely broad ($>10^\circ\text{C}$) TNZs given their small size, and seemingly have a $T_{uc} > 40^\circ\text{C}$. As such a large-scale physiological investigation of hyperthermic torpor is lacking and we have yet to reach consensus as to whether or not heterotherms could enter torpor at high T_a . Reaching a consensus precludes any attempts to understand the putative benefits of hyperthermic torpor or how it could affect a species' response to climate change (Nowack et al. 2017). The data presented in Chapter 4 seeks to provide the corroborating physiological evidence to support the idea that heterotherms can down-regulate metabolism at T_a s typically associated with hyperthermia.

A meta-analysis on the thermoregulatory profiles of 29 bat species showed that relative to homeothermic bats, heterothermic bats maintain lower T_b at similar T_a but display similar upper limits (T_{uc}) of TNZ and similar costs of evaporative water loss. Crucially, T_{uc} in heterotherms exceeds their T_b . Based on the principles of heat exchange (Arrhenius 1915, Scholander et al. 1950, McNab 1980, Geiser 1988, Lloyd & Taylor 1994, Schmidt-Nielsen 1997, Tattersall 2012), heterotherms could not have achieved this without some form of metabolic down-regulation. Importantly, unlike at low T_a , it may not be possible for animals to display metabolic rates lower than basal metabolism at high T_a because of the opposing influence of the Arrhenius effect forcing an increase in metabolism. By down-regulating metabolism at high T_a , heterotherms can alter their heat balance by lowering the contribution of their endogenous heat production that is associated with metabolic activity. By doing so, heterotherms would reduce their total heat burden and slow the rate of heat storage at moderate T_a or prevent dehydration at high T_a ; hence delay the onset of severe heat stress. The data further suggests that heterotherms tend to endure moderate heat stress to conserve water to achieve $T_b < T_a$ at more extreme T_a . Thus, heterotherms seem to have a significant physiological advantage over homeotherms when dealing with heat and are thus more likely to survive the effects of global warming.

The ability of heterotherms to use hyperthermic torpor also has significant implications for models attempting to predict species' responses to global warming and forecast likely distributions. It is generally accepted that species will respond to the current anthropogenically driven climate change with a compensatory migration poleward or towards higher altitude (Walther et al. 2002, Root et al. 2003, Parmesan 2006). This view is based on species distribution models (synonymous with climate-envelope models, niche models or bioclimatic models) which rely on the principle of niche conservatism, that is, that species will retain the ecological characteristics of their ancestor and should seek to remain within similar niches

(Wiens & Graham 2005, Dawson et al. 2011, Diamond et al. 2012). However, given the availability of microclimates within which species can minimize their exposure to environmental perturbations and, in combination with the use of hyperthermic torpor by heterotherms, many of the currently accepted predictions of species responses may deviate from what is actually observed.

The evolution of endothermy in mammals

There is no obvious reason why adaptive heterothermy seems to have been lost in tarsiers. Nevertheless, there was no evidence of adaptive heterothermy in the population of *C. bancanus* studied here or in other studies involving *Tarsius syrichta* (McNab & Wright 1987, Lovegrove et al. 2014). It is possible that the lack of adaptive heterothermy in tarsiers may be due to the low sample sizes of relevant studies ($n \leq 4$ per study), or as is the case in *Galago moholi* (Nowack et al. 2010), tarsiers may potentially use torpor under extreme conditions only, or adaptive heterothermy may truly be lost.

Brain tissue is energetically costly (Aiello & Wheeler 1995, Isler & van Schaik 2006, 2009) and the ability of tarsiers to leap between trees and successfully hunt at night requires highly derived visual acuity, depth perception and neuronal accompaniment (Castenholz 1984, Collins et al. 2005, Isler & van Schaik 2009). Even though tarsiers appear to reduce the costs associated with enhanced sensory perception through a morphological readjustment in the brain (Stephan 1984, Schwartz 2003, Grabowski et al. 2016) and a reduction in costly splanchnic tissue (Aiello & Wheeler 1995), the cost associated with their brain and nocturnal habits could still preclude the use of torpor. Alternatively, with the exception of some, for example monotremes (McAllan and Geiser, 2014), marsupials (Geiser et al. 2008) and bats (Stawski et al. 2014), reproduction and adaptive heterothermy appear mutually exclusive in mammals (Lee et al. 1990, Mzilikazi & Lovegrove 2002, Levesque & Lovegrove 2014). Indeed, tarsiers have long gestation periods of ca. 5-6 months and, in the case of *C. bancanus*, breed throughout the year (MacKinnon & MacKinnon 1980, Wright et al. 1986). Thus, as is argued for *G. moholi* (Mzilikazi et al. 2006, Nowack et al. 2010), the perpetually high level of reproductive hormones associated with their continued reproductive readiness and gestation could preclude the use of torpor. Though, the most likely explanation for the lack of adaptive heterothermy in tarsiers would involve some combination of factors related to their costly brains, maintaining reproductive readiness, and their development. In comparison to the adult size, tarsier neonates have relatively large brains and large body sizes (Izard et al. 1985, Roberts 1994). Thus, although tarsiers benefit by having large neonatal brains due to rapid behavioural development, the cost associated with this benefit is a slow postnatal growth rate (Roberts 1994) and long gestation period.

Given their phylogenetic placement as basal in the Haplorrhini (Hartig et al. 2013), the thermoregulatory capacity of tarsiers provide crucial insight and context for the evolution of endothermy in the rest of the haplorrhines (Lovegrove 2012, 2017). Although one cannot rule out the potential for adaptive heterothermy within the Tarsiidae, the apparent loss of adaptive heterothermy in *C. bancanus* and *T. syrichta*, as well as the lack of evidence for adaptive heterothermy in other haplorrhines such as *Callithrix jacchus* (Petry et al. 1986), *C. pygmaea* (Genoud et al. 1997), *Saguinus oedipus* (Stonerook et al. 1994) and *Callimico goeldii* (Kälin et al. 2003, Power et al. 2003), suggests that adaptive heterothermy may have been lost either in the ancestor of the tarsiers or in the tarsier clade. The significance of the absences of adaptive heterothermy in haplorrhines bares implications regarding their future survivability and the possibility of humans to travel to deep space in induced hyperthermic states.

Limitations of the current study and recommendations for future work

A potential technical limitation of the thermoregulatory work conducted in the current thesis is that the water vapour was removed from the incurrent airstream before reaching the study animals. Even though the intention of removing water vapour from the incurrent air was to provide study animals with a favourable gradient for heat dissipation to minimize the risk of fatality due to heat stress, given the high humidity of their environment, the dry air would have been an unnatural experience for them. Thus, there is the potential that the thermoregulatory data, especially that of the rate of evaporative water loss, may have been negatively influenced by the dry air entering the respirometers (but see Withers & Cooper 2014). Another technical limitation of the thesis was the low sample size and fairly short period of observation of thermoregulatory data in free-ranging animals. The failure of several of the temperature sensitive data-loggers that were implanted into tarsiers severely limited our ability to make inferences of their thermoregulatory capacity and precluded a more compelling argument for the lack of adaptive heterothermy in tarsiers. Nonetheless, the current thesis argues that adaptive heterothermy may be lost in haplorrhines. Given the significance of the thermoregulatory capacity of tarsiers, future studies should endeavour to augment existing data. Alternatively, given that there are 177 species in the clade Haplorrhini, future work could also endeavour to assess the occurrence of adaptive heterothermy in other members of the clade. A good starting point would be to assess the occurrence of adaptive heterothermy in the 11 anthropoid species listed in Chapter 3, or in any of the other species of tarsier.

It would also be beneficial for future studies to augment our dataset of thermoregulatory variables for bats. An augmented dataset would allow us to further explore the interplay between evaporative water loss and metabolism to better understand the interspecific variations between the onset of evaporative water loss. This could potentially

answer the question of why the onset of evaporative cooling occurs well before TNZ in some species (Hosken & Withers 1997, Marom et al. 2006) or at T_{lc} in others (Hosken & Withers 1999, Cooper & Withers 2012), yet metabolism remains the same throughout TNZ irrespective of whether EWL is low or moderate. With the benefit of an augmented dataset, future studies could also redo the analyses presented in Chapter 4 to support the findings presented in the current thesis. Alternatively, future studies could adopt the techniques described in Chapter 4, and repeat the procedure using another group of animals. As mentioned in Chapter 4, such studies could focus on marsupials as hyperthermic torpor has been claimed to occur in the grey short-tailed opossum (*Monodelphis domestica*; Busse et al. 2014) and eastern pygmy-possum (*Cercartetus nanus*; Song et al. 1997) or possibly rodents, due to the claim of hyperthermic torpor in spiny mice (*Acomys russatus*, Grimpo et al. 2013). In addition, future studies should also attempt a direct determination of metabolic down-regulation. While doing so, it would be fruitful to simultaneously determine the concentration of heat shock proteins, 5' adenosine monophosphate-activated protein kinase (AMPK), or pyruvate dehydrogenase kinase, to assess their usefulness as marker enzymes of hyperthermic torpor (Hardie and Hawley, 2001; Hardie et al., 2003; Melvin and Andrews, 2009; Storey et al., 2010; Mihaylova and Shaw, 2011; Storey and Storey, 2011).

Lastly, any improvement in our understanding of thermoregulation and other physiological tolerances also serves to better inform studies geared towards modelling species response to global warming. Given the uncertainty of the rate and magnitude of global warming, as well as confounding effects such as microhabitats and phenotypical flexibility, future studies must adopt a mechanistic approach to determine species' physiological tolerances. Such an understanding provides a framework for conservation efforts because tolerances provide a robust limit of what species can endure and thus their chances of future survival.

Literature cited

- Aiello, L.C., and Wheeler, P. (1995). The Expensive-Tissue Hypothesis: The brain and the digestive system in human and primate evolution. *Curr. Anthropol.* 36(2), 199-221. doi: <http://www.jstor.org/stable/2744104>.
- Arrhenius, S. (1915). *Quantitative laws in biological chemistry*. London: Bell.
- Bondarenko, A., Körtner, G., and Geiser, F. (2014). Hot bats: extreme thermal tolerance in a desert heat wave. *Naturwissenschaften* 101(8), 679-685. doi: 10.1007/s00114-014-1202-2.
- Busse, S., Lutter, D., Heldmaier, G., Jastroch, M., and Meyer, C.W. (2014). Torpor at high ambient temperature in a neotropical didelphid, the grey short-tailed opossum

- (*Monodelphis domestica*). *Naturwissenschaften* 101(11), 1003-1006. doi: 10.1007/s00114-014-1226-7.
- Castenholz, A. (1984). "The eye of *Tarsius*," in *Biology of tarsiers*, ed. C. Niemitz. (Stuttgart: Gustav Fischer Verlag), 303-318.
- Chevin, L.M., Lande, R., and Mace, G.M. (2010). Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLOS Biol* 8(4), e1000357. doi: 10.1371/journal.pbio.1000357.
- Chown, S.L., Hoffmann, A.A., Kristensen, T.N., Angilletta, M.J., Stenseth, N.C., and Pertoldi, C. (2010). Adapting to climate change: A perspective from evolutionary physiology. *Clim. Res.* 43(1-2), 3-15. doi: 10.3354/cr00879.
- Collins, C.E., Hendrickson, A., and Kaas, J.H. (2005). Overview of the visual system of tarsius. *Anat Rec A Discov Mol Cell Evol Biol* 287A(1), 1013-1025. doi: 10.1002/ar.a.20263.
- Cooper, C.E., and Withers, P.C. (2012). Does season or captivity influence the physiology of an endangered marsupial, the numbat (*Myrmecobius fasciatus*)? *J Mammal* 93(3), 771-777. doi: 10.1644/11-MAMM-A-084.2.
- Dawson, T.P., Jackson, S.T., House, J.I., Prentice, I.C., and Mace, G.M. (2011). Beyond predictions: Biodiversity conservation in a changing climate. *Science* 332(6025), 53-58. doi: 10.1126/science.1200303.
- Diamond, S.E., Nichols, L.M., McCoy, N., Hirsch, C., Pelini, S.L., Sanders, N.J., et al. (2012). A physiological trait-based approach to predicting the responses of species to experimental climate warming. *93*(11), 2305-2312. doi: 10.1890/11-2296.1.
- Gaveau, D.L.A., Sheil, D., Husnayaen, Salim, M.A., Arjasakusuma, S., Ancrenaz, M., et al. (2016). Rapid conversions and avoided deforestation: examining four decades of industrial plantation expansion in Borneo. *Sci Rep* 6, 32017. doi: 10.1038/srep32017
- <https://www.nature.com/articles/srep32017#supplementary-information>.
- Geiser, F. (1988). Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? *J Comp Physiol B* 158(1), 25-37. doi: 10.1007/bf00692726.
- Geiser, F., Christian, N., Cooper, C.E., Körtner, G., McAllan, B.M., Pavey, C.R., et al. (Year). "Torpor in marsupials: recent advances", in: *Hypometabolism in animals: torpor, hibernation and cryobiology, 13th international hibernation symposium*, eds. B.G. Lovegrove & A.E. McKechnie: University of KwaZulu-Natal, Pietermaritzburg), 297-306.
- Genoud, M., Martin, R.D., and Glaser, D. (1997). Rate of metabolism in the smallest simian primate, the pygmy marmoset (*Cebuella pygmaea*). *Am. J. Primatol.* 41(3), 229-245.

- Grabowski, M., Voje, K.L., and Hansen, T.F. (2016). Evolutionary modeling and correcting for observation error support a 3/5 brain-body allometry for primates. *J. Hum. Evol.* 94, 106-116. doi: 10.1016/j.jhevol.2016.03.001.
- Grimpo, K., Legler, K., Heldmaier, G., and Exner, C. (2013). That's hot: golden spiny mice display torpor even at high ambient temperatures. *J Comp Physiol B* 183(4), 567-581. doi: 10.1007/s00360-012-0721-4.
- Hardie, D.G., and Hawley, S.A. (2001). AMP-activated protein kinase: the energy charge hypothesis revisited. *BioEssays* 23(12), 1112-1119. doi: 10.1002/bies.10009.
- Hardie, D.G., Scott, J.W., Pan, D.A., and Hudson, E.R. (2003). Management of cellular energy by the AMP-activated protein kinase system. *FEBS Letters* 546(1), 113-120. doi: [http://dx.doi.org/10.1016/S0014-5793\(03\)00560-X](http://dx.doi.org/10.1016/S0014-5793(03)00560-X).
- Hartig, G., Churakov, G., Warren, W.C., Brosius, J., Makalowski, W., and Schmitz, J. (2013). Retrophylogenomics place tarsiers on the evolutionary branch of anthropoids. *Sci. Rep.* 3, 1756. doi: 10.1038/srep01756.
- Hosken, D.J., and Withers, P.C. (1997). Temperature regulation and metabolism of an Australian bat, *Chalinolobus gouldii* (Chiroptera: Vespertilionidae) when euthermic and torpid. *J Comp Physiol B* 167(1), 71-80. doi: 10.1007/s003600050049.
- Hosken, D.J., and Withers, P.C. (1999). Metabolic physiology of euthermic and torpid lesser long-eared bats, *Nyctophilus geoffroyi* (Chiroptera: Vespertilionidae). *J Mammal* 80(1), 42-52. doi: 10.2307/1383206.
- Isler, K., and van Schaik, C.P. (2006). Metabolic costs of brain size evolution. *Biol. Lett.* 2(4), 557-560. doi: 10.1098/rsbl.2006.0538.
- Isler, K., and van Schaik, C.P. (2009). The Expensive Brain: A framework for explaining evolutionary changes in brain size. *J. Hum. Evol.* 57(4), 392-400. doi: 10.1016/j.jhevol.2009.04.009.
- Izard, M.K., Wright, P.C., and Simons, E.L. (1985). Gestation length in *Tarsius bancanus*. *Am. J. Primatol.* 9(4), 327-331. doi: 10.1002/ajp.1350090408.
- Jacobs, M. (1988). *The tropical rain forest: A first encounter*. Berlin: Springer-Verlag.
- Kälin, N., Martin, R.D., and Genoud, M. (2003). Basal rate of metabolism and temperature regulation in Goeldi's monkey (*Callimico goeldii*). *Comp. Biochem. Phys. A* 135(2), 279-290. doi: [http://dx.doi.org/10.1016/S1095-6433\(03\)00077-1](http://dx.doi.org/10.1016/S1095-6433(03)00077-1).
- Karlsson, I.M. (2000). Nocturnal air temperature variations between forest and open areas. *J Appl Meteorol* 39(6), 851-862.
- Kearney, M.R., Wintle, B.A., and Porter, W.P. (2010). Correlative and mechanistic models of species distribution provide congruent forecasts under climate change. 3(3), 203-213. doi: 10.1111/j.1755-263X.2010.00097.x.

- Laurance, W.F. (2004). Forest-climate interactions in fragmented tropical landscapes. *Philos Trans R Soc Lond B Biol Sci* 359(1443), 345-352. doi: 10.1098/rstb.2003.1430.
- Lee, T.M., Pelz, K., Licht, P., and Zucker, I. (1990). Testosterone influences hibernation in golden-mantled ground squirrels. *Am J Physiol* 259(4 Pt 2), R760-R767. doi: 10.1152/ajpregu.1990.259.4.R760.
- Levesque, D.L., and Lovegrove, B.G. (2014). Increased homeothermy during reproduction in a basal placental mammal. doi: 10.1242/jeb.098848.
- Lloyd, J., and Taylor, J.A. (1994). On the temperature dependence of soil respiration. *Funct Ecol* 8(3), 315-323. doi: 10.2307/2389824.
- Lovegrove, B.G. (2012). The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol Rev* 87(1), 128-162. doi: 10.1111/j.1469-185X.2011.00188.x.
- Lovegrove, B.G. (2017). A phenology of the evolution of endothermy in birds and mammals. *Biol Rev* 92, 1213-1240. doi: 10.1111/brv.12280.
- Lovegrove, B.G., Canale, C., Levesque, D.L., Fluch, G., Rehakova-Petru, M., and Ruf, T. (2014). Are tropical small mammals physiologically vulnerable to arrhenius effects and climate change? *Physiol Biochem Zool* 87(1), 30-45. doi: 10.1086/673313.
- MacKinnon, J., and MacKinnon, K. (1980). The behavior of wild spectral tarsiers. *Int. J. Primatol.* 1(4), 361-379. doi: 10.1007/bf02692280.
- Maloney, S.K., Bronner, G.N., and Buffenstein, R. (1999). Thermoregulation in the Angolan free-tailed bat *Mops condylurus*: A small mammal that uses hot roosts. *Physiol Biochem Zool* 72(4), 385-396. doi: 10.1086/316677.
- Marom, S., Korine, C., Wojciechowski, M., Tracy, C.R., and Pinshow, B. (2006). Energy Metabolism and Evaporative Water Loss in the European Free-Tailed Bat and Hemprich's Long-Eared Bat (Microchiroptera): Species Sympatric in the Negev Desert. 79(5), 944-956. doi: 10.1086/505999.
- McAllan, B.M., and Geiser, F. (2014). Torpor during reproduction in mammals and birds: Dealing with an energetic conundrum. *Integr Comp Biol* 54(3), 516-532. doi: 10.1093/icb/icu093.
- McNab, B.K. (1980). On estimating thermal conductance in endotherms. *Physiol. Zool.* 53(2), 145-156. doi: 10.1086/physzool.53.2.30152577.
- McNab, B.K. (1989). Temperature regulation and rate of metabolism in 3 Bornean bats. *J Mammal* 70(1), 153-161. doi: 10.2307/1381678.
- McNab, B.K., and Wright, P.C. (1987). Temperature regulation and oxygen consumption in the Philippine tarsier *Tarsius syrichta*. *Physiol. Zool.* 60(5), 596-600. doi: 10.2307/30156133.

- Melvin, R.G., and Andrews, M.T. (2009). Torpor induction in mammals: recent discoveries fueling new ideas. *Trends Endocrinol Metab* 20(10), 490-498. doi: 10.1016/j.tem.2009.09.005.
- Mihaylova, M.M., and Shaw, R.J. (2011). The AMP-activated protein kinase (AMPK) signaling pathway coordinates cell growth, autophagy, & metabolism. *Nat Cell Biol* 13(9), 1016-1023. doi: 10.1038/ncb2329.
- Mzilikazi, N., and Lovegrove, B.G. (2002). Reproductive activity influences thermoregulation and torpor in pouched mice, *Saccostomus campestris*. 172(1), 7-16. doi: 10.1007/s003600100221.
- Mzilikazi, N., Masters, J.C., and Lovegrove, B.G. (2006). Lack of torpor in free-ranging southern lesser galagos, *Galago moholi*: ecological and physiological considerations. *Folia Primatol.* 77(6), 465-476. doi: 10.1159/000095392.
- Nowack, J., Mzilikazi, N., and Dausmann, K.H. (2010). Torpor on demand: heterothermy in the non-lemur primate *Galago moholi*. *PLoS ONE* 5(5), e10797. doi: 10.1371/journal.pone.0010797.
- Nowack, J., Stawski, C., and Geiser, F. (2017). More functions of torpor and their roles in a changing world. *J Comp Physiol B* 187(5), 889-897. doi: 10.1007/s00360-017-1100-y.
- Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Systemat.* 37, 637-669. doi: 10.2307/30033846.
- Petry, H., Riehl, I., and Zucker, H. (1986). Energieumsatzmessungen an Weißbüscheläffchen (*Callithrix jacchus*). *J. Anim. Physiol. Anim. Nutr.* 55(1-5), 214-224. doi: 10.1111/j.1439-0396.1986.tb00722.x.
- Power, M.L., Tardif, S.D., Power, R.A., and Layne, D.G. (2003). Resting energy metabolism of Goeldi's monkey (*Callimico goeldii*) is similar to that of other callitrichids. *Am. J. Primatol.* 60(2), 57-67.
- Ricklefs, R.E., and Wikelski, M. (2002). The physiology/life-history nexus. *Trends Ecol. Evol.* 17(10), 462-468. doi: 10.1016/s0169-5347(02)02578-8.
- Roberts, M. (1994). Growth, development, and parental care in the western tarsier (*Tarsius bancanus*) in captivity: Evidence for a "slow" life-history and nonmonogamous mating system. *Int. J. Primatol.* 15(1), 1-28. doi: 10.1007/BF02735232.
- Rodríguez-Durán, A. (1995). Metabolic rates and thermal conductance in four species of neotropical bats roosting in hot caves. *Comp Biochem Phys A* 110(4), 347-355. doi: [http://dx.doi.org/10.1016/0300-9629\(94\)00174-R](http://dx.doi.org/10.1016/0300-9629(94)00174-R).
- Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C., and Pounds, J.A. (2003). Fingerprints of global warming on wild animals and plants. *Nature* 421(6918), 57-60. doi: 10.1038/nature01333.

- Schmidt-Nielsen, K. (1997). *Animal physiology: adaptation and environment*. Cambridge (UK): Cambridge University Press.
- Scholander, P.F., Hock, R., Walters, V., Johnson, F., and Irving, L. (1950). Heat regulation in some arctic and tropical mammals and birds. 99(2), 237-258.
- Schwartz, J.H. (2003). "How close are the similarities between *Tarsius* and other primates," in *Tarsiers: past, present, and future*, eds. P.C. Wright, E.L. Simons & S. Gursky. (New Brunswick: Rutgers University Press), 50-96.
- Sherwood, S.C., and Huber, M. (2010). An adaptability limit to climate change due to heat stress. *Proc. Natl. Acad. Sci. U. S. A.* 107(21), 9552-9555. doi: DOI 10.1073/pnas.0913352107.
- Song, X., Körtnier, G., and Geiser, F. (1997). Thermal relations of metabolic rate reduction in a hibernating marsupial. *Am J Physiol Regul Integr Comp Physiol* 273(6), R2097-R2104.
- Stawski, C., Willis, C.K.R., and Geiser, F. (2014). The importance of temporal heterothermy in bats. *J Zool* 292(2), 86-100. doi: 10.1111/jzo.12105.
- Stephan, A. (1984). "Morphology of the brain in *Tarsius*," in *Biology of tarsiers*, ed. C. Niemitz. (Stuttgart: Gustav Fischer Verlag), 317-344.
- Stonerook, M.J., Weiss, H.S., Rodriguez-M, M.A., Rodríguez-M, J.V., Hernández-C, J.I., Peck, O.C., et al. (1994). Temperature-metabolism relations in the cotton-top tamarin (*Saguinus oedipus*) model for ulcerative colitis. *J. Med. Primatol.* 23(1), 16-22. doi: 10.1111/j.1600-0684.1994.tb00090.x.
- Storey, K.B., Heldmaier, G., and Rider, M.H. (2010). "Mammalian hibernation: physiology, cell signaling, and gene controls on metabolic rate depression," in *Dormancy and Resistance in Harsh Environments*, eds. E. Lubzens, J. Cerda & M. Clark. (Berlin, Heidelberg: Springer Berlin Heidelberg), 227-252.
- Storey, K.B., and Storey, J.M. (2011). Heat shock proteins and hypometabolism: adaptive strategy for proteome preservation. *Res Rep Biol* 2, 57-68.
- Tattersall, G.J. (2012). "Diurnal Changes in Metabolic Rate in Pygmy Marmosets: Implications for Sleep, Torpor, and Basal Metabolism in Primates," in *Living in a Seasonal World*. Springer), 471-480.
- Tattersall, G.J., Sinclair, B.J., Withers, P.C., Fields, P.A., Seebacher, F., Cooper, C.E., et al. (2012). Coping with thermal challenges: Physiological adaptations to environmental temperatures. *Compr. Physiol.* 2, 2151-2202. doi: 10.1002/cphy.c110055.
- Tuff, K.T., Tuff, T., and Davies, K.F. (2016). A framework for integrating thermal biology into fragmentation research. *Ecol Lett* 19, 361-374. doi: 10.1111/ele.12579.
- Turner, I., and Corlett, R.T. (1996). The conservation value of small, isolated fragments of lowland tropical rain forest. 11(8), 330-333.

- Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., et al. (2002). Ecological responses to recent climate change. *Nature* 416(6879), 389-395. doi: 10.1038/416389a.
- Wiens, J.J., and Graham, C.H. (2005). Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu. Rev. Ecol. Evol. Systemat.* 36(1), 519-539. doi: 10.1146/annurev.ecolsys.36.102803.095431.
- Withers, P.C., and Cooper, C.E. (2014). Physiological regulation of evaporative water loss in endotherms: is the little red kaluta (*Dasykaluta rosamondae*) an exception or the rule? *Proc R Soc B* 281(1784). doi: 10.1098/rspb.2014.0149.
- Wolf, B.O., Coe, B.H., Gerson, A.R., and McKechnie, A.E. (2017). Comment on an analysis of endotherm thermal tolerances: systematic errors in data compilation undermine its credibility. *Proc R Soc B* 284:20162523(1855). doi: 10.1098/rspb.2016.2523.
- Wright, P.C., Izard, M.K., and Simons, E.L. (1986). Reproductive cycles in *Tarsius bancanus*. *Am. J. Primatol.* 11(3), 207-215. doi: 10.1002/ajp.1350110302.